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12TH WORLD CONGRESS ON ANIMAL, PLANT AND MICROBIAL TOXINS

Cuernavaca, Mexico
September 21-26, 1997

This Congress took place in Cuernavaca, Morelos, Mexico. Approximately 320 participants from 30 different countries were present. The program included seven plenary lectures, six mini-symposia, 87 oral presentations in free communications sessions and 167 poster presentations. Only the plenary lectures and symposia will be covered here. The abstracts of the entire Congress will be printed by *Toxicon*, the official journal of the International Society on Toxinology (it is currently in press).

TOXINS AS POTENTIAL DRUGS

Animal toxins are powerful tools that can be used in pharmacology and in functional analysis. The first plenary lecture was presented by **DR. ANDRE MENEZ** (CEA/Saclay, Gif-Sur-Yevette, France) who gave an overview of the functional architecture of many toxic proteins and their potential use for drug design. The three dimensional structure of many toxins have been elucidated. All this information has helped in the understanding of the diversity of toxin functions. There are at least three distinct situations. First, a group of venomous animals produces multiple toxins with the same fold, suggesting functional divergence on a conserved structural theme. Second, animals from distinct phyla produce toxins with similar functions but different folds, suggesting a functional convergence of the folds. Third, animals from different phyla produce toxins with similar functions and fold, suggesting both structural and functional convergences. In addition, toxin folds are present in various tissues other than venom glands and even in plants, where they usually exert quite distinct functions. All this information can be used now to understand the molecular evolution of many animal toxins and could also be used in the engineering of new functions and in the designing of novel drugs.

A symposium on the same subject presented some interesting uses of animal toxins.

DR. ALAN L. HARVEY (University of Strathclyde, Glasgow, U.K.) used two neurotoxins: dendrotoxins and muscarinic toxins, to analyze the role of K^+ channels and muscarinic receptors in changes of neuronal properties with aging and cognition. By using displacement studies with dendrotoxins demonstrated the selective lost of Kv1.1 channels in the hippocampus and septohippocampal pathway with aging and by using muscarinic toxin MT3 demonstrated the role of M4 muscarinic receptors in cognition.

DR. LOURIVAL D. POSSANI (National University of Mexico, Cuernavaca, Mexico) reviewed data on Noxiustoxin (a toxic peptide

for K^+ channels). The expertise generated during the study of Noxiustoxin served as a model for the design and assay of Shiva-3 (a synthetic peptide similar to cecropins). Shiva-3 inhibits, in the midgut of mosquitoes, the development of the sporogonic phase of *Plasmodium berghei* (responsible for a murine type of malaria). The Shiva-3 gene was constructed and expressed as a hybrid protein, in which Glutathione-S-transferase was placed at the N-terminal part and Shiva-3 at the C-terminal region of the recombinant. The idea is to develop knowledge for the production of transgenic mosquitoes capable of secreting Shiva-3 or similar peptides in the midgut of mosquitoes, in an attempt to control the spreading of malaria.

DR. PASCALE MARCHOT (CNRS - Jean Roche Institut, Marseille, France) studied fasciculin which is a member of the three fingered toxins that inhibit acetylcholinesterase (AChE). The crystal structure of the complex fasciculin-acetylcholinesterase (Fas2-mouse AChE) was presented and showed that this toxin totally occludes the substrate entry into the catalytic site of AChE, principally by hydrophobic interactions of loops 2 and 3. A synthetic gene that codes Fas2 was prepared and expressed. Site-directed mutagenesis of this gene was produced in 16 distinct amino acid positions and studied in this context (Fas2-AChE interactions).

DR. MICHAEL GUREVITZ (Tel-Aviv University, Israel) used the anti-insect scorpion neurotoxins to develop novel bioinsecticides compatible with the environment. Scorpion genes have been modify in order to have mutants with elevated toxicity and with improved insect selectivity. These genes have been used in the construction of transgenic baculoviruses that could help in the control of important insect pests as *Heliothis armigera* and *Spodoptera littoralis*. Special effort was invested studying the yellow scorpion toxin "LqhalT". This strategy could be used for the control of other pest as cockroach.

Finally, **DR. ROBERT R. LUTHER** group (Neurex Corporation, Menlo Park, CA, USA) presented non clinical data obtained with a synthetic peptide corresponding to "omega"-conotoxin from snails of the genus *Conus*, that selectively blocks mammalian N-type neuronal voltage-sensitive Ca^{2+} channels. This toxin can be used as a powerful pharmacotherapeutic agent since by blocking these channels a variety of intracellular Ca^{2+}-dependent process could be controlled as neuronal excitability, release of neurotransmitters, activation of second messenger systems and gene transcription. Preliminary studies demonstrated that "omega"-conotoxin posses analgesic and neuroprotective properties, hence could be used in the treatment and management of pain and in ischemic brain injury.

PHOSPHOLIPASE A2 TOXINS

A symposium on phospholipase A2 toxins started with the presentation of **DR. CHARLOTTE L. OWNBY** (Oklahoma State

University, Stillwater OK, USA). She mentioned that phospholipases A2 (PLA2) constitute a family of toxins that have similar three dimensional structures but many different functions as neurotoxicity, myotoxicity, anticoagulant and proinflamatory activities. They have been classified in three groups according to their primary structure. Type I include enzymes from mammalian pancreas and human spermatozoa and venoms from some snakes (Elapidae and Hydrophidae family). Type II includes enzymes in the venoms of other snakes (Viperidae family and sub-families Crotalinae and Viperinae) and some mammalian cells (platelets, gastric mucosae and vascular endothelium). Type III are enzymes from Mexican beaded lizard, European honeybee and Gila monster. Her group have been studying the myotoxic function of different PLA2 in muscle cells. All three types of PLA2 have this activity.

The myotoxin activity of PLA2s was particularly studied by **DR. JOHN B. HARRIS** (University of Newcastle, Newcastle upon Tyne, U.K.) and **DR. JEFFREY E. FLETCHER** (Allegheny University of the Health Center, Philadelphia, USA). The work of Dr. Harris is focused at the elucidation of the mechanism of action of phospholipase myotoxins. This is a multistep process. First the toxin bind to an acceptor located in the plasma membrane of the muscle fiber, however this acceptor has not been identified. The association of the toxin and the lipids leads to an hydrolysis of the membrane lipids producing an increase in membrane fluidity and lost of ion gradients. These steps are followed by the hydrolysis of key components in the cytoskeleton that result in the disruption of the sarcomers allowing the enzymatic degradation of the major contractile proteins actin and myosin. It is not known which parts of the toxins are directly responsible of myotoxicity and which are the enzymes activated to breakdown the functional proteins of skeletal fibers.

Dr. Jeffrey E. Fletcher showed that there are two different mechanisms by which the phospholipase A2 myotoxins lyse cells. The first one works at lower concentrations of PLA2 (0.1-0.5 μM) and requires long incubations periods. This activity is antagonized by methylprednisolone and correlates with myotoxicity. The second mechanism works at high concentrations of PLA2 (1-5 μM) incubated over short periods.

Other PLA2 activities were also analyzed as the anticoagulant effect and the neurotoxic activity. **DR. CASSIAN BON** (Pasteur Institute, Paris, France) showed that some PLA2 have other activities such as anticoagulant effect. This activity has been found in PLA2 from snake venoms and also in some human PLA2 such as blood platelets and macrophages. Other venom PLA2 inhibit prothrombinase activity, by possibly binding to factor Va or Xa. The anticoagulant effect is independent from the enzymatic activity of the enzyme and this activity has been mapped to be a region comprising residues 54-77. Furthermore, a peptide corresponding to residues 51-74 of human platelet PLA2 mimics the action of whole PLA2 on factor Xa.

The neurotoxic activity of Trimucrotoxin was analyzed by **DR. INN-HO TSAI** (Institute of Biological Chemistry, Taipei, Taiwan) which

performed site-directed mutagenesis of this PLA2 from *Trimeresurus mucrosquamatus* venom in order to localize the site responsible for neurotoxic activity. The analysis of a collection of mutants in Asn6 demonstrated that this highly conserved residue in conjunction with residues 7 and 8 could be important for the binding with the neuronal binding sites.

The capacity to disrupt lipid membranes by the catalytically inactive PLA2 mutant Asp49Lys was presented by **DR. RAGHUVIR ARNI** (Department of Physics, UNESP, Sao José do Rio Preto, Brazil). The structure of crystalline dimers of this PLA2 was determined and a spectroscopic study of the different dimer conformations was performed by analysis of changes in the intrinsic fluorescence emission of single tryptophan residues located at the dimer interface. Significant differences in the relative orientation of the monomers have been observed resulting in open and closed dimer conformations, that would permit an access to the lipid binding and catalytic sites.

DR. FRANC GUBENSEK (University of Ljubljana, Slovenia) presented an interesting evolutionary analysis of PLA2, using two myotoxic analogues as model: ammodytoxin C and ammodytin L. Typical genomic organization of these PLA2 consist of 5 exons separated by 4 introns. An ART-2 retroposon sequence was found in the 4th intron of the PLA2 genes. This transposon DNA elements inserts into the genome and remains stable, functioning as an evolutionary marker. The Bov-B line found in ruminants were also found in viperids. The degree of sequence divergence indicate their amplification during the Mesozoic era in Squamata, about 100 million years ago.

Finally, an illuminating plenary lecture on PLA2 toxins was presented by **DR. MICHAEL LAZDUNSKY** (CNRS Institute of Molecular and Cellular Pharmacology, Valbonne, France). His talk was focused on the PLA2 family of receptors. The large spectrum of PLA2 effects are apparently linked to the existence of a variety of very high affinity receptors. The N-type receptors were firstly isolated from brain tissues and were shown to have high affinity for the neurotoxic PLA2 and are made of proteins of 36-51 and 85 kDa. They have been found in many other tissues. The M-type receptors have been cloned and sequenced from rabbit and human muscle cells. The M-type receptor is a 180 kDa glycoprotein that has 24% identity with the mannose receptor of macrophages. It is a membrane protein with a N-terminal cysteine rich domain, a fibronectin type II domain, eight repeats of a carbohydrate recognition domain, a unique transmembrane domain and an intracellular C-terminal domain. This receptor has a high affinity for the venom PLA2 but also for the mammalian secretory PLA2, indicating that receptors for toxic PLA2 are the normal targets of endogenous PLA2. Mutagenesis of PLA2 demonstrated that residues within or close to the Ca^{2+} binding loop are important in the binding to the receptor. The M-type receptor has been also analyzed by site-directed mutagenesis. It was found

that repeat number 5 of the carbohydrate recognition domain and Lys35 are involved in the interaction with the PLA2 toxin. The role of the receptor in PLA2 internalization was discussed.

STRUCTURE AND FUNCTION OF SEVERAL TOXINS

Animal toxins have been used as probes for the elucidation of the structure and function of ion channels. **DR. MARIA L. GARCIA** (Merck Research Laboratories, Rahway, NJ, USA) presented a plenary lecture related to the use of scorpion toxins to study K^+ channels. Charybdotoxin, one of the most widely study scorpion toxins, binds to the outer vestibule of certain K^+ channels and blocks the ion conductance by physical occlusion of the pore. This toxin has been used for purification of the smooth muscle high conductance (250 pSiemens) Ca^{2+} activated K^+ channels, for the determination of channel subunits composition (alpha and beta subunits) and for further development of the channel pharmacology. The role of the voltage gated K^+ channel (Kv1.3) in human T-lymphocytes signal transduction process was analyzed by using margatoxin and noxiustoxin. Both toxins are able to block the Kv1.3, inhibit the T-lymphocyte activation response and the lymphokin production. They have a role in immunosuppression and may be a powerful tool in the treatment of autoimmune diseases as well as transplantation therapy.

A special symposium on this subject took place, starting with the presentation by **DR. GEORGE K. CHANDY** (University of California, Irvine, CA, USA). He used four different scorpion toxins: charybdotoxin (ChTX), kaliotoxin (KTX), margatoxin (MgTX) and noxiustoxin (NTX) to determine the architecture of the P-region of the K^+ channel Kv1.3. All toxins bind to the channel, and only the binding of ChTX could be inhibited by protonation (changing the pH). An Arg25 residue of this toxin was used to analyze the interaction with His residues located in the Kv1.3 channel. This analysis showed that the channel has a shallow (8 Å deep), 30 Å wide, saucer-shaped vestibule at the external entrance to the pore. This information was used to select a Stichoclactyle (ShK) toxin mutant that selectively blocks Kv1.3 K^+ channel activity with a much lower effect on the Kv1.1 K^+ channels. Mutant K22 from ShK toxin blocks Kv1.3 channel, while being >100 fold less effective on related channels. This new drug could be used as an immunosuppressant.

In this symposium new toxins were described. **DR. MURIEL DELEPIERRE** (Pasteur Institute, Paris, France) presented the resolution of the three dimensional structure of a novel toxic peptide (Pi1) purified from the venom of the scorpion *Pandinus imperator*, which toxin is able to block the *Shaker*-B K^+ channels, expressed in Sf9 cells. The structure was determined by homonuclear proton 2D NMR methods at 500 MHz, using a nanoprobe (only 50 nmoles of toxin!). This peptide has a short alpha-helix (Ser8-Thr18) and a double-stranded beta-sheet plate, stabilized

by two disulfide bridges, out of the four present in Pi1. The two antiparallel strands of the beta-sheet structure extends from Asn22 to Cys32 with a tight turn at Arg28-Met29 in contact with the N-terminal fragment Leu1 to Cys4.

DR. RAYMOND S. NORTON (Biomolecular Research Institute, Parkville, Australia) presented the resolution of two toxins resolved by ^{1}H-NMR spectroscopy. The omega-conotoxin GVIA, which blocks N-type Ca^{2+} channels, consists of a small triple-stranded beta-sheet and a disulfide knot. Alanine scanning by mutagenesis of this toxin have been used in order to identify residues important for binding to the Ca^{2+} channels. Mutants in residues at Tyr13 and Lys2 affect the binding to channels. The three dimensional structure of the anemone ShK toxin, a potent blocker of K^+ channel Kv1.3, was also obtained. This toxin consist of two helices and a series of turns, showing a structure not related to the structure of the known scorpion toxins that interact with the same channel

DR. HERVÉ ROCHAT presented results obtained with maurotoxin, another K^+-channel specific toxin, purified from *Scorpio maurus*. This toxin is a basic and C-terminal amidated 34 amino acid residue polypeptide cross linked by four disulfide bridges. The location of these bridges was determined by Edman degration analysis on a sample of synthetic maurotoxin. The disulfide pairing is different from that of Pi1 described by Muriel Delepierre. However, synthetic maurotoxin blocks the Kv1.1, Kv1.2 and Kv1.3 K^+ channels in a similar way as the natural maurotoxin, which makes it an interesting model for structure-function studies of K^+-channel specific toxins.

DR. R. MANJUNATHA KINI is involved in the identification of protein-protein interaction sites. Many factors affect protein interactions (shape complementary, electrostatic and hydrogen bonds and buried hydrophobic sites). He analyzed the amino acids that are flanking binding sites of proteins and found that proline residues are highly abundant. The functional sites for calciseptine, a L-type calcium channel blocker, were searched. A putative fragment located between Pro42 and Pro47 was identified.

Finally, and still in the subject of arthropod toxins, **DR. EUGENE GRISHIN** (Shemyakin and Ovchinnikov Institute of Bioorganic Chemistry, Moscow, Russia) presented a plenary lecture regarding the present and future of toxins isolated from the black widow spider venom. Alpha-latrotoxin provokes a massive transmitter release from different nerve endings of vertebrates, whereas delta-latrotoxins seems to be specific for insects and crustaceans. The cDNA genes that code for these toxins were cloned and the amino acid sequences analyzed. These toxins are polypetides of approximately 1000 amino acids. Several domains of these proteins were analyzed, including the ankrin-like repeats. These toxic-proteins can form ion channels. In *Xenopus* oocytes they open permeable pores for Ca^{2+}, with conductances between 3-200 pS. Attempts to characterize and isolate the alpha-latrotoxin

receptor were performed. The putative receptor has been identified as latrophilin (190 kDa).

IMMUNOTHERAPY

DR. CASSIAN BON (Pasteur Institute, Paris, France) chaired a symposium on immunotherapy. In the field of toxinology is means the utilization of antivenoms, different serum and/or antibody preparations capable of producing neutralization of toxins or venoms. **DR. JEAN-PHILIPPE CHIPPAUX** (Research Center for Meningitis and Schistosomiasis, Niamey, Nigeria) showed the development of immunotherapy to control snake envenomations in many African tropical countries. In Africa the annual incidence of snakebites is in the order of 1 million and the mortality rate about 50,000 persons per year. The utilization of highly purified IgG fragments, injected intravenously, leads to a best tolerance and higher efficacy of immunotherapy on snakebites. Also the utilization of an adjuvant therapy permits the reduction of the doses of antivenom to be injected into patients, and allows to increase the efficiency while reducing its cost.

DR. JULIAN WHITE (Women's and Children's Hospital, North Adelaide, Australia), contribution (presented in absence by Dr. Dietrich Mebs) was focused on spider bites and insect stings envenomations in Australia. From the total number of accidents spider bites represent 53.7%, insect stings 39.3%, marine animals 4% and snakebites only the 3% of the cases. Snakebites affect from 1000 to 3000 people per year, with an annual mortality of only 2 cases. Up to 500 cases require snake antivenoms each year. A venom detection kit (based on ELISA analysis) is used in order to identify the type of antivenom required, instead of using a polyvalent antivenom. Also, a premedication (based on the administration of antihistamine and adrenaline) prior to antivenom is proposed. There is a highly frequent incidence of black spider bites; between 5,000-10,000 cases, annually. The antivenom used is 94% effective and is applied in 20% of the cases.

DR. JOSÉ MARIA GUTIERREZ (Clodomiro Picado Institute, San Jose, Costa Rica) presented a study on the development and use of horse antivenoms against the crotaline snakes in Central America. The local tissue damage represents a serious consequence of crotaline and viperine snake envenomations. It encompasses a complex series of alterations, including hemorrhage, edema, myonecrosis and dermonecrosis. Due to its fast development it is extremely difficult to neutralize by antivenoms if there is a delay on the onset of serotherapy application. Thus, the administration of antivenoms with a high antibody titer is a key element in this treatment. He found that equine whole IgG and F(ab)2 antivenoms show similar efficacy in the neutralization of edema, myonecrosis and hemorrhage induced by *Bothrops asper* venom.

DR. VALERIE CHOUMET (Pasteur Institute, Paris, France)

presented results aimed at the optimization of antivenom therapy following viper bites in France. An ELISA was developed in parallel with a clinical grading scale based on the earlier characteristic signals of envenoming. A good correlation was observed between venom levels in the blood and clinical symptoms. The antivenom kinetics was studied and a redistribution of the venom was observed from the extravascular compartment to the vascular one, where it is sequestered by the antibodies. Intravenously injection was shown to be the most effective route for antivenom administration. Finally, she showed that F(ab)2 antibodies were more effective than Fab in detoxification of *Vipera aspid* envenomations in rabbits.

DR. LINEU FREIRE-MAIA (Federal University of Minas Gerais, Belo Horizonte, Brazil) presented data on the treatment of patients stung by scorpions, in Brazil. An ELISA system was used to detect venom antigens. In rats, a fast absorption of venom from subcutaneous tissue, a fast distribution to other tissues and a very slow elimination process was found. Treatment of 18 patients presenting systemic manifestations of envenoming, with 5-30 ml of antivenom (Fab'2 fragment, 7 g/100 ml protein content) over 10 min., cleared the high levels of venom detected in plasma 1 h after the treatment. Also vomiting and local pain decreased after 1 h. At 12 h after treatment the hyperglycemia was not detected and the cardiorespiratory manifestations disappeared after 6-24 h. The mortality rate was 0.28% in 3,860 patients treated.

DR. MOHAMMAD ISMAIL (King Fahad National Guard Hospital, Riyadh, Saudi Arabia) presented the pharmacokinetics of ^{125}I-labeled IgG, F(ab')2 and Fab fractions of scorpion and snake antivenins into model animals: rabbit and mouse. The F(ab')2 possessed the fastest disposition rate constants and the shortest distribution half-lives, while Fab showed the lowest disposition rate constant and the longest distribution half-lives. The venom distribution is much faster than antibodies, however the F(ab')2 fraction is the best to rescue the animals from envenomation rendering it as the most suitable to be used in serotherapy of scorpion and snake envenomations.

DR. RICHARD DART (Rocky Mountain Poison and Drug Center, Denver, CO, USA) presented the utilization of polyespecific ovine Fab antibodies for the treatment of crotalid snakebite in USA. Two clinical trials were conducted with humans stung by snakes. His results show that polyespecific ovine Fab is safe and effective. Repeated antivenom dosing appears preferable to single dose therapy. In addition, re-emergence of coagulation abnormalities occurs regularly after North American rattlesnake bites.

FOOD POISONING

Animals may acquire toxicity by absorbing toxic compounds from their food. Sequestration and accumulation of toxins occurs

in some species and could provide protection against predators. **DR. DIETRICH MEBS** (University of Frankfurt, Frankfurt, Germany) presented examples of this food poisoning pathway, by which toxins such as ciguatoxin from marine dinoflagellate, palitoxin from zoanthids and microcystin from blue-green algae of fresh water can enter the food poisoning chain. Insects provide an interesting model to study the mechanism of toxin resistance and dissemination of toxic compound in the food.

DR. TAKESHI YASUMOTO (Tohoku University, Japan) presented the results of structural configuration analysis of maitotoxin and described a novel toxin carchatoxin. He reviewed several cases of human intoxication in the Pacific Ocean area, due to ciguatoxin, maitotoxin and palitoxin which are initially accumulated in herbivorous and then carnivorous fishes. When the contaminated fishes are eaten by humans cause intoxication and quite often dead.

The ciguatera poisoning in Baja California Sur - Mexico, was also studied by **DR. ARTURO P. SIERRA-BELTRAN** (The Center for Biological Research, La Paz, Mexico). His group has isolated liposoluble toxins from the fishes *Mycteroperca priomura* and *Lutjanus colorado*. The isolated toxins were evaluated on mouse bioassays, that developed ciguatera symptoms. The toxin was identified as ciguatoxin-1 by HPLC analysis.

DR. RYUICHI HIGUCHI (Kyushu University, Fukuoka, Japan) presented the structure and biological activities of different compounds obtained from *Opisthobranchia* and soft corals. From the first ones new isoprenoids, halogenated monoterpenoids, halogenated acetogenins, sesterpenoids and spongian diterpenoids were isolated and characterized. Some terpenoids show *in vitro* cytotoxic activity against lymphoma L1210 cells and against human epidermoid carcinoma KB cells. From soft coral they have isolated new eunicellin type, xenicane-type, cembrane-type and amphilectane-type diterpenoids that display toxicity to brine shrimp.

DR. GEOFFREY A. CODD (University of Dundee, Dundee, Scotland) presented an overview of cyanobacterial toxins that are hazardous to human and animal health. About 60 cyanobacterial toxins have been identified, including hepatotoxins, neurotoxins and cytotoxins. He discussed the need for precautionary principles and presented approaches to anticipate and reduce the adverse effects of these toxins.

MICROBIAL AND PLANT TOXINS

A plenary lecture was presented by **DR. GERHARD HABERMEHL** (Hannover School of Veterinary Medicine, Hannover, Germany) on plant toxins. The secondary metabolites are products not essential for the daily life metabolism of plants. They are thought to be substances for protection against consumption by other organisms, because of their burning or bitter taste. These toxins belong to different classes: alkaloids usually act as

neurotoxins, steroid-glycosides as cardiotoxins and terpenes as substances affecting the complete intestinal system including liver and kidney. Terpenes are also known to inhibit or promote plant growth. The tertiary metabolites are toxins derived for external sources. One example is the shrub *Baccharis cordifolia* that receive the toxin from the fungus *Myrothecium verrucaria*.

An additional symposium was conducted by Dr. Leonard Smith and Robert J. Carman on Microbial Toxins.

DR. ROBERT J. CARMAN (TechLab, Inc., Blacksburg, VA, USA) talked about *Clostridium perfringens* toxins. This bacteria produces many extracellular toxins. Some are enterotoxic and cause diarrhea (enterotoxin "beta", "epsilon" and "tau"), some are enzymes (neuraminidase, "alpha" is a phospholipase C, "mu" is a hyaluronidase and "tau" is an ADP ribosyl transferase). The enterotoxin binds to a 50 kDa receptor in the human plasma membrane and then the complex binds to a 70 kDa protein. After binding the cellular permeability is affected causing diarrhea. Susceptible hosts range from humans, domestic livestock and laboratory, zoo and wild animals. They can be treated with vancomycin and metronidazole.

DR. FREDERICK J. CASSELS (Walter Reed Army Institute of Research, Washington, USA) presented a strategy to produce vaccines against the diarrheagenic enterotoxigenic *Escherichia coli* (ETEC) based primarily on virulence determinats. The bacteria produce colonization factors in the surface, also called adhesins, allowing the initial attachment and colonization. These colonization factors were purified and cloned (4 genes operon) in order to be produced and microencapsulated into biodegradable microespheres to obtain a safe, efficacious, inexpensive and multivalent oral vaccine. Clinical trials with the microencapsulated CS6 are currently being planned.

DR. THOMAS L. HALE (Walter Reed Army Institute of Research, Washington, USA) talked about *Shigella* vaccines. Shigelosis accounts for 20% of diarrheal disease and 50% of dysentery. Three different vaccine programs were analyzed. First a live, genetically attenuated *S. flexneri 2a*, that has been successfully tested for safety and immunogenicity in adult (104 doses). Second, an acid hydrolyzed *S. sonnei* lipopolysaccharide (LPS), chemically conjugated to a protein carrier (the idea is to introduce this conjugated in a mutant *Pseudomonas* affected in exotoxin production). Finally, the third vaccine is a meningococcal outer membrane protein vesicles with non-covalent associated *S. sonnei* LPS, shown to elicit serum and intestinal antibody response when administered intranasally.

DR. ROBERT G. ULTRICH (U.S. Army Medical Research Institute of Infectious Disease, Frederick-MD, USA) presented the development of bacterial superàntigen vaccines that could protect against many bacteria. Superantigens are soluble proteins expressed by many staphylococcal and streptococcal bacteria, that bind to the human MHC class II molecule, HLA-DR and T-cell

antigen receptors. These vaccines stimulate the immune system producing a high-long lived protective antibody levels.

DR LEONARD SMITH (U.S. Army Medical Research Institute of Infectious Disease, Frederick-MD, USA) showed recent advances on the development of genetically engineered vaccines for botulinum neurotoxins. Botulinum is one of the most powerful bacterial toxins. This research group have produced synthetic genes encoding non-toxic, carboxy-terminal region (Hc) of botulinum toxin. These genes were introduced in yeast and their products were expressed intra- and extra-cellularly. The extracellular product is glycosylated. Gene products were analyzed for protection in mice and proven to be effective.

HISTORICAL EVENTS OF OUR INTERNATIONAL SOCIETY ON TOXINOLOGY.

PROFESSOR FINDLAY E. RUSSEL (University of Arizona, Tucson-AZ, USA), one of the founders of our IST-society was kind to accept the invitation to give a lecture on the founding history of our Society. Names and facts of the founders and historical events were shown and commented by Dr. F. Russel.

THIS SUMMARY WAS PREPARED BY:

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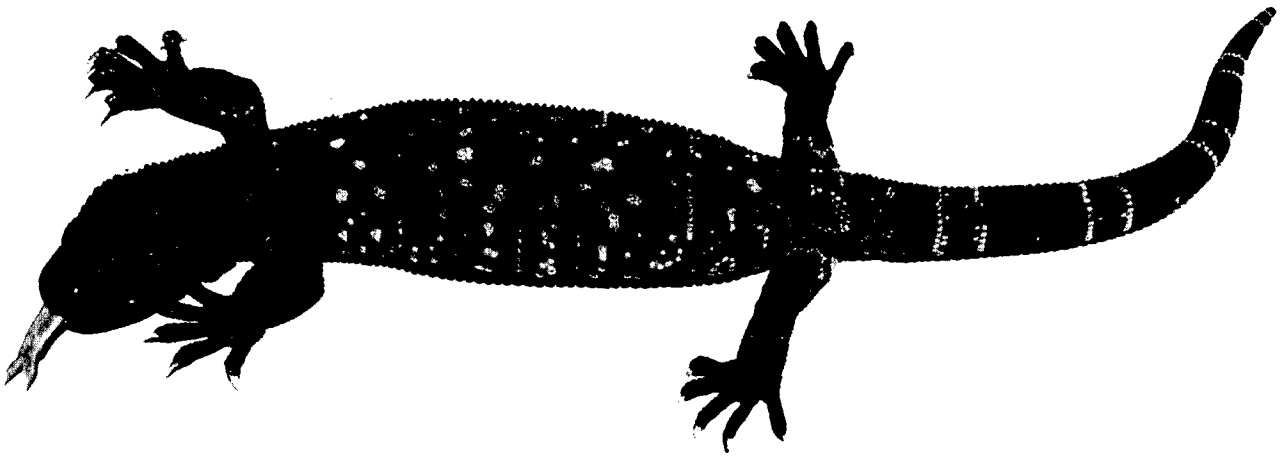
INTERNATIONAL SOCIETY ON TOXINOLOGY

12th World Congress on Animal, Plant and Microbial Toxins



Sunday, September 21, to friday 26, 1997

Cuernavaca-México

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NATIONAL AUTONOMOUS UNIVERSITY OF MEXICO

12TH WORLD CONGRESS ON ANIMAL, PLANT AND MICROBIAL TOXINS
International Society on Toxinology

SPONSORED BY THE BIOTECHNOLOGY INSTITUTE OF THE NATIONAL AUTONOMOUS UNIVERSITY OF MEXICO, IN CUERNAVACA, STATE OF MORELOS, MEXICO

Sunday, September 21, to Friday September 26, 1997

NOTE ON THE COVER FIGURE: The picture of the cover page of the booklet of the congress was taken by Sergio Trujillo from a *Heloderma horridum horridum*, known by the trivial name of Mexican Beaded Lizard. This is one of three only species of venomous lizards of the world. The geographical distribution of this animal is restricted to the Pacific Coast of Mexico. The United States (USA) species, from the Desert of Arizona, is *Heloderma suspectum*, also known by the common name of Gila Monster. These animals are in danger of extinction.

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SUBJECT INDEX

- ***HOW TO USE THIS BOOK**: Abbreviations & Symbols/General Schedule*

1. Sunday is **Su**, Monday is **Mo**, Tuesday is **Tu**, Wednesday is **We**, Thursday is **Th** and Friday is **Fr**.

2. The congress is divided into four distinct types of activities: **Pl** means plenary lectures, **S** means symposium presentation, **O** means oral presentation and **P** means poster presentation.

3. Each one of these activities have a corresponding number, for example: Pl-01Mo, means plenary lecture number 1 to be presented on Monday; S-04Mo, means symposium talk number 4 to be presented on Monday; O-88Fr, means oral presentation number 88 to be presented on Friday; P-123Tu, means poster presentation 123 (this number will be labeled on top of the board where the text of the abstract, figures and illustrations must be posted), to be presented on Tuesday. Since these numbers serve to show the pagination of the booklet, no page numbers are given on the abstracts section.

4. All these symbols are indicated in the small box on top of each abstract in the section of abstracts. The total number of each activity is indicated on page 2, and follows the order: Pl, S, O and finally P.

4. The general program (pages 8-11) gives an over-all view of the entire congress and it starts with roman number **I** (see also page 2). The specific program, with detailed titles and authors are initiated by **II**. The abstracts of the congress are included on section **III**, whereas the author index is on **IV**.

5. A general map of the hotel and of the location of the auditorium (Salon ALHAMBRA) and the corresponding rooms are included on pages 6 and 7. Location of events are indicated also in the Specific Scientific Program section (pages 12-42).

6. The posters should be mounted on the panel indicated, according to their respective numbers, from 08:30 in the morning to the end of the day activity (for example, on Tuesday it finishes at 20:00). You can started posting from 07:30 in the morning if you wish. The author (co-authors) must be present in front of the poster during the poster hours (consult detailed program for the exact timing, usually from 14:00 to 16:00).

General Schedule:

The congress starts with a short opening ceremony on Sunday, 21th of September at 19:00. It ends on Friday the 26th of September at 16:15 (Consult: I-General Program, or II-Specific scientific program, for detailed schedule of events).

GENERAL ORGANIZATION

A. - LOCAL ORGANIZING COMMITTEE

- Lourival D. Possani, Ph.D.
  President of the 12th IST Congress

- Alejandra Bravo, Ph.D.
  Editorial Office

- Eduardo Gallegos, C.P.
  Treasurer

- Maria Canela, Fredy Coronas and Fernando Zamudio,
  Operational support

B. - INTERNATIONAL ORGANIZING COMMITTEE
(alphabetical order, family name)

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- Alejandra BRAVO - Mexico
- Julia Prado FRANCESCHI - Brazil
- Gerhard HABERMEHL - Germany
- Dietrich MEBS- Germany
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- Charlotte OWNBY - U.S.A.
- Lourival D. POSSANI - Mexico
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- Leonard SMITH - U.S.A.
- David TEAKSTON - Great Britain

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- *INTERNATIONAL*

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5TH PAN-AMERICAN SYMPOSIUM IST - 1995, FREDERICK, MD, USA
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- ***NATIONAL*** *(Mexico)*

INSTITUTE OF BIOTECHNOLOGY - UNAM
COORDINATION OF THE SCIENTIFIC RESEARCH - UNAM
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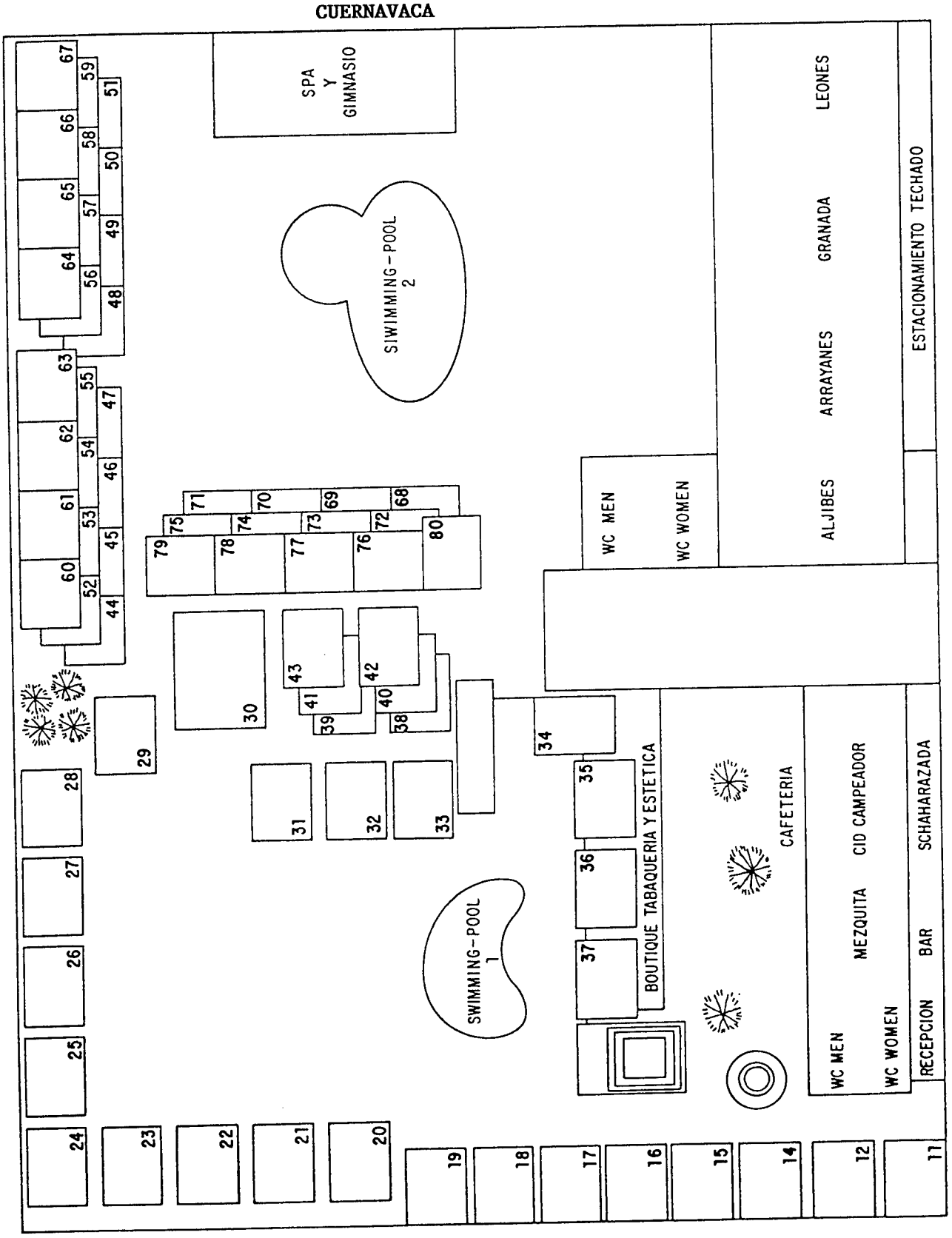
GENERAL MAP OF THE HOTEL VILLA BEJAR
CUERNAVACA



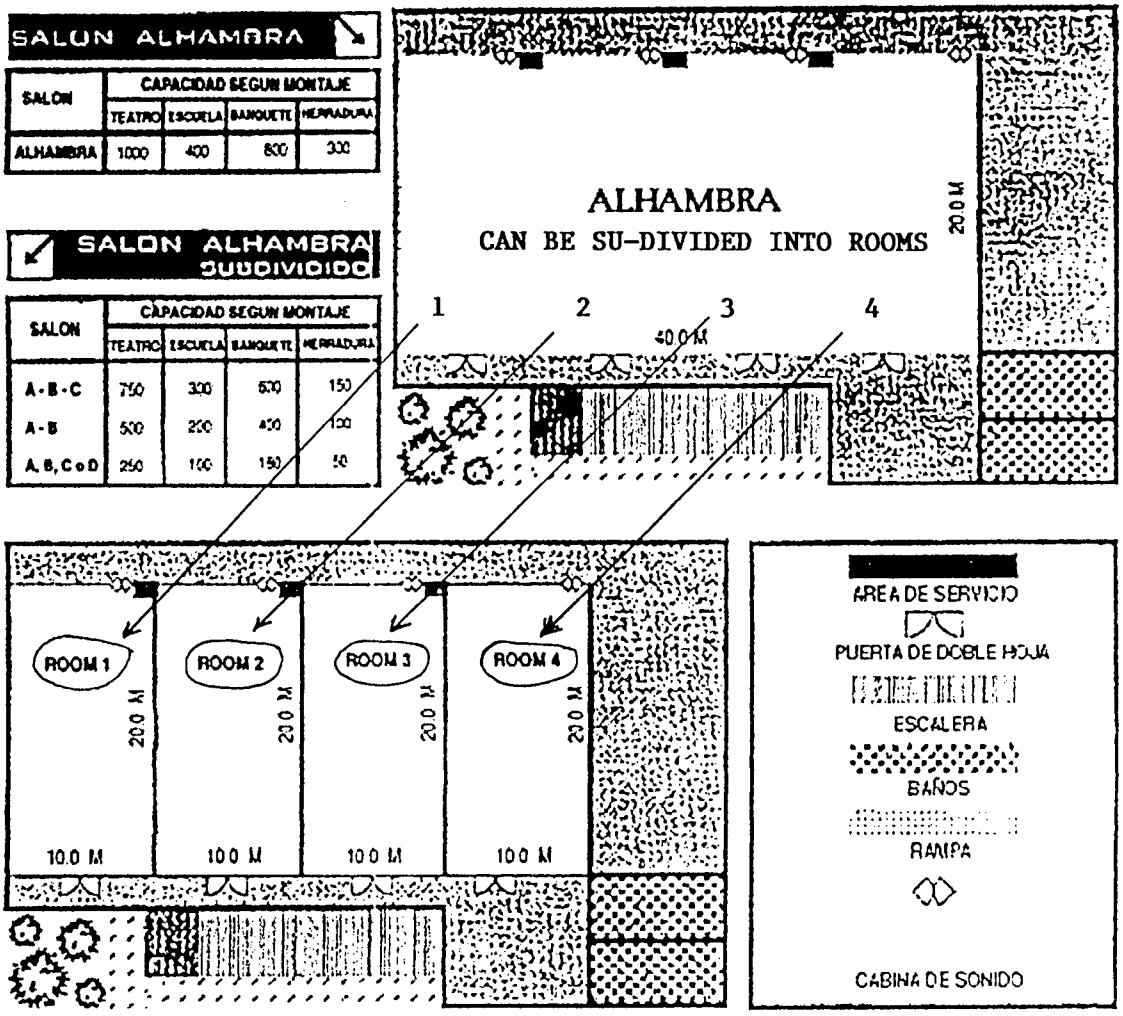
HOTELES VILLA BEJAR
cuernavaca

SALON ALHAMBRA

SALON	CAPACIDAD SEGUN MONTAJE			
	TEATRO	ESCUELA	BANQUETE	HERRADURA
ALHAMBRA	1000	400	800	300

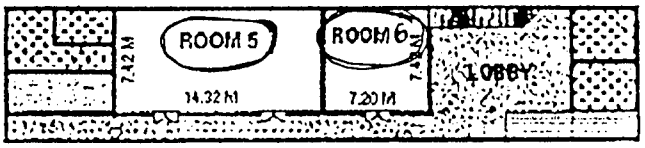
SALON ALHAMBRA SUBDIVIDIDO

SALON	CAPACIDAD SEGUN MONTAJE			
	TEATRO	ESCUELA	BANQUETE	HERRADURA
A-B-C	750	300	600	150
A-B	500	200	400	100
A, B, C o D	250	100	150	50



SALON MEZQUITA Y CID CAMPEADOR

SALON	CAPACIDAD SEGUN MONTAJE			
	TEATRO	ESCUELA	BANQUETE	HERRADURA
CID CAMPEADOR	80	48	70	32
MEZQUITA	20	16	10	15



CIRCLE SHOW THE ROOMS OF THE CONGRESS: NUMBERS 1,2,3,4,5 AND 6

I.- GENERAL PROGRAM

I - GENERAL PROGRAM

SUNDAY, 21 SEPTEMBER 1997

REGISTRATION, RECEPTION AND INFORMATION DESK. 8:00 to 20:00

OPENING CEREMONY 19:00
Salon Alhambra (Rooms 1 and 2 - see brochure of Hotel)

Opening Address
Dr. Lourival D. Possani, President of the 12th World Congress on Animal, Plant and Microbial Toxins

Welcome
Dr. Charlotte Ownby, President of the International Society on Toxinology

Inauguration
Dr. Francisco Barnés de Castro, President of the National Autonomous University of Mexico

Welcome cocktail
In the area labeled "Cafeteria" (see brochure)

MONDAY, 22TH SEPTEMBER 1997

REGISTRATION, RECEPTION
AND INFORMATION DESK. 8:30 to 13:00; 14:00 to 20:00

GENERAL NOTE REGARDING LOCALIZATION OF EVENTS: All lectures and symposia will take place at Salon Alhambra (rooms 1 and 2, shown in the brochure of the hotel). Oral presentations will take place in Rooms 1 plus 2, 3 of Salon Alhambra, and Room 5 = Cid Campeador. All Poster Sections will take place either in Room 3 or 4, or both, from Salon Alhambra - see brochure)

FIRST PLENARY LECTURE

08:30-09:30 **Dr. André Ménez:** "*Functional architectures of toxic proteins : A clue for drug design?* (PL-01Mo)

SYMPOSIUM NUMBER 1: "PHOSPHOLIPASE A_2 TOXINS"

09:35-12:25 Please refer to specific program below (within the symposium a refreshment break from 10:35-10:50)

12:25-14:00 - **LUNCH** (on own)

14:00-16:00 - **POSTER SECTION ON PURIFICATION, STRUCTURE, ELECTROPHYSIOLOGY AND FUNCTIONAL PROPERTIES, ROOMS 3,4 (See list in the detailed scientific program below)**

16:00-17:00 - **INITIAL PART OF TWO SIMULTANEOUS ORAL PRESENTATIONS ON PURIFICATION AND STRUCTURE, ROOMS 1,2 (See list in the detailed scientific program below)**

17:00-17:20 - Refreshment Break

17:20-18:50 - **FINAL PART OF TWO SIMULTANEOUS ORAL PRESENTATIONS ON PURIFICATION AND STRUCTURE, ROOMS 1,2 (See list in the detailed scientific program below)**

18:50-19:00 - INTERVAL

SECOND PLENARY LECTURE

19:00-20:00 - **Dr. Findlay Russel:** "*Founding History of the International Society on Toxinology*" (PL-02Mo)

20:00-20:30 - Business meeting of the International Society on Toxinology (Salon Alhambra - Rooms 1 and 2)

TUESDAY, 23TH SEPTEMBER 1997

REGISTRATION, RECEPTION
AND INFORMATION DESK. 8:30 to 13:00; 14:00 to 20:00

THIRD PLENARY LECTURE

08:30-09:30 **Dr. Maria L. Garcia:** "*Scorpion toxins: tools for studying K^+ channels*" (PL-03Tu)

SYMPOSIUM NUMBER 2: "TOXINS AS POTENTIAL DRUGS"

09:35-12:25 Please refer to specific program below (within the symposium a refreshment break from 10:35-10:55)

12:25-14:00 - **LUNCH** (on own) - Editorial Board of Toxicon meeting during lunch - Salon Herradura - Room 6)

14:00-16:00 - **POSTER SECTION MOSTLY ON IMMUNOTHERAPY AND CLONING (See list in the detailed scientific program below)**

16:00-18:50 - **TWO SIMULTANEOUS ORAL PRESENTATIONS: IMMUNOTHERAPY I, ACTION OF ION-CHANNELS AND INVERTEBRATE TOXINS** (within the oral presentation a refreshment break from 17:00-17:20), (See list in the detailed scientific program below)

18:50-19:00 - INTERVAL

FOURTH PLENARY LECTURE

19:00-20:00 - **Dr. Michel Lazdunsky:** "*A family of receptor for venom phospholipase A_2*" (PL-04Tu)

WEDNESDAY, 24TH SEPTEMBER 1997

REGISTRATION, RECEPTION
AND INFORMATION DESK. 8:30 to 13:00; 14:00 to 20:00

SYMPOSIUM NUMBER 3: "FOOD POISONING"

08:30-11:00 Please refer to specific program below

11:00-11:15 - Refreshment Break

FIFTH PLENARY LECTURE

11:15-12:15 *REDI AWARD LECTURE (PERSON TO BE NOMINATED)*
(No abstract available)

12:15-20:00 - **BOX-LUNCH FOR TRIPS, VISITS OR JUST RELAX**

20:00 - DINNER OF THE CONGRESS

21:00-22:20 - FOLKLORIC BALLET OF THE STATE OF MORELOS

THURSDAY, 25TH SEPTEMBER 1997

REGISTRATION, RECEPTION
AND INFORMATION DESK. 8:30 to 13:00; 14:00 to 20:00

SIXTH PLENARY LECTURE

08:30-09:30 **Dr. Eugene Grishin:** "*Black widow spider toxins: the present and the future*" (PL-05Th)

SYMPOSIUM NUMBER 4: "IMMUNOTHERAPY"

09:35-12:25 - Please refer to specific program below

12:25-14:45 - **LUNCH WITH POSTER SECTION ON BIOASSAYS, BIOLOGICAL EFFECTS, MICROBIAL TOXINS AND OTHERS**
(*See list in the detailed scientific program below*)

SYMPOSIUM NUMBER 5: "STRUCTURE AND FUNCTION OF SEVERAL TOXINS"

14:45-17:15 - Please refer to specific program below

17:15-17:30 - Refreshment Break

17:30-20:15 - **THREE SIMULTANEOUS ORAL PRESENTATIONS: BIOLOGICAL EFFECTS, INTERACTION OF VENOM COMPONENTS WITH THEIR RECEPTORS AND IMMUNOTHERAPY II, ROOMS 1,2 AND 5**
(*See list in the detailed scientific program below*)

FRIDAY, 26TH SEPTEMBER 1997

RECEPTION AND INFORMATION DESK. 8:30 to 13:00; 14:00 to 17:00

SEVENTH PLENARY LECTURE

08:30-09:30 **Dr. Gerhard Habermehl:** "*Secondary and tertiary metabolites of plant toxins*" (PL-06Fr)

SYMPOSIUM NUMBER 6: "MICROBIAL TOXINS"
09:35-12:25 - Please refer to specific program below

12:25-14:00 - **LUNCH** (on own)

14:00-16:15 - **TWO SIMULTANEOUS ORAL PRESENTATIONS ON CLONING AND BIOLOGICAL EFFECTS III, ROOMS 1,2**
(See list in the detailed scientific program below)

16:15 - CLOSING CEREMONY

II.- SPECIFIC SCIENTIFIC PROGRAM

II - SPECIFIC SCIENTIFIC PROGRAM - (*divided into sections A to E*)

MONDAY, 22th SEPTEMBER, 1997

A - FIRST PLENARY LECTURE

08:30-09:30 **Dr. André Ménez:** "*Functional architectures of toxic proteins : A clue for drug design?* (PL-01Mo)

B - SYMPOSIUM NUMBER 1: "PHOSPHOLIPASE A_2 TOXINS"
Chairperson: Dr. Charlotte L. Ownby
Co-chairperson: Dr. Michel Lazdunski

09:35-09:55 - Phospholipase A_2 toxins: diversity in structure and function, by Charlotte L. Ownby, Terry R. Colberg and Heloisa S. Selistre-de-Araújo. (S-01Mo)
09:55-10:15 - Skeletal muscle damage caused by myotoxic phospholipases, by John B. Harris, R. Dixon, R. Vater, A. Faiz and D. Mantle. (S-02Mo)
10:15-10:35 - Lys49 phospholipase A_2 myotoxins lyse cell cultures by two distinct mechanisms, by Jeffrey E. Fletcher and Ming-shi-Jiang.(S-03Mo)
10:35-10:50 - Refreshment Break
10:50-11:10 - Anticoagulant phospholipase A_2: from snake venoms to human enzymes, by C. Mounier and Cassian Bon. (S-04Mo)
11:10-11:30 - At the interface-crystal structures of phospholipase A_2s, by Raghuvir Arni and R. J. Ward. (S-05Mo)
11:30-11:50 - Effect of site-directed mutagenesis on the activity of recombinant Trimucrotoxin, a neurotoxic phospholipases from *Trimeresurus mucrosquamatus* venom, by Inn-Ho Tsai and Y. M. Wang. (S-06Mo)
11:50-12:10 - The Bov-B line found in *Vipera ammodytes* toxic PLA_2s is widely spread in snake genomes, by Franc Gubensek and D. Kordis. (S-07Mo)
12:10-12:25 - Discussion

12:25-14:00 - **LUNCH** (on own) - Editorial Board of Toxicon meeting during lunch - Room 6 - Salon Herradura)
14:00-16:00 - **POSTER SECTION** (See below, letter E)

C - ORAL PRESENTATIONS

ROOM 1: *SUBJECT MOSTLY RELATED TO STRUCTURE*
Chairperson: Dr. Paul Fletcher Jr.
Co-chairperson: Dr. P. Gopalakrishnakone

16:00-16:15 - Non-RGD disintegrin-like/cysteine rich-domains of Atrolysin A: inhibition of platelet aggregation by recombinant

protein and peptide antagonists. Li-Guo Jia, Xiao-Ming Wang, John D. Shannon, Jon B. Bjarnason and Jay W. Fox. (O-01Mo)

16:15-16:30 - Neurotoxin receptors sites on sodium channels - who interacts with who and how? Dalia Gordon. (O-02Mo)

16:30-16:45 - A probabilistic approach to find functionally significant loci in sequences of peptidic toxins. Carlos Sevcik and Gina D'Suze. (O-03Mo)

16:45-17:00 - Probing for the involvement of asparagine 67 in substrate recognition of *Trimeresurus flavoviridis* venom phospholipase A_2 by protein engineering and molecular dynamics. Tomohisa Ogawa, Hidehiro Tou, Satoru Kuhara, Kouji Muramoto and Motonori Ohno. (O-04Mo)

17:00-17-20 - Refreshment break

17:20-17:35 - Newly identified K^+ channel toxins from *Tityus sp.* and *Centruroides sculpturatus* scorpions. P. L. Fletcher Jr., B. M. Martin, L. J. Bartolotti, M. Fletcher, S. Lucas, D.D. Watt, L.K. Fainter, G.A. White and M. Mannie. (O-05Mo)

17:35-17:50 - NMR structure of a T epitope-containing cyclic peptide with restricted conformations and increased neutralizing antibody responses against the toxin O from *Naja nigricollis* snake. Jean-Marc Lancelin, Isabelle Krimm, Michel Léonetti, Joël Cotton and André Ménez. (O-06Mo)

17:50-18:05 - Chemical reactivity of charybdotoxin: an electronic structure based point of view. Joel Ireta and Marcelo Galván. (O-07Mo)

18:05-18:20 - Identification and functional study of an acetylcholine receptor-mimicking antibody/short-chain toxin complex as revealed by molecular modelling and mutational analysis. Frédéric Ducancel, Nathalie Germain, Karine Mérienne, Sophie Zinn-Justin, Jean-Claude Boulain and André Ménez. (O-08Mo)

18:20-18:35 - The NMR studies of cardiotoxins from Taiwan cobra (*Naja naja atra*): Structure, dynamics, folding and interaction. Chin Yu. (O-09Mo)

18:35-18:50 - Modelling of Acanthoxin, a PLA2 from the venom of the Australian death adder. Peter G. Hains, Paul A. Ramsland and Kevin W. Broady. (O-10Mo)

18:50-19:00 - INTERVAL

19:00.20:00 - SECOND PLENARY LECTURE (See below, letter D)

ROOM 2: *SUBJECT MOSTLY RELATED TO PURIFICATION AND FUNCTION*
Chairperson: Dr. Ivan Kaiser
Co-chairperson: Dr. George Odell

16:00-16:15 - Crotoxin subunit cross-linking with a chemically cleavable reagent. I.I. Kaiser, C. Seebart, K. Spencer and M. Reis. (O-11Mo)

16:15-16:30 - Identification of allergenic components in snake venom by human antivenom IgE. L.G.D. Heneine and S.S. Gontijo. (O-12Mo)

16:30-16:45 - Isolation of protocoagulant protein from bristles of *Lonomia obliqua* caterpillars. A. M. Chudzinski-Tavassi, C. V. Reis, L. R. C. Gonçalves and E.M.A. Kelen. (O-13Mo)

16:45-17:00 - Components and characteristics of *Grammastola cala* venom, the beautiful tarantula of Chili. G.V.Odell, M.P.Doss, A. W. Fenton, S.A. Hudiburg, C.L. Ownby and J.N. Mills. (O-14Mo)

17:00-17-20 - Refreshment break

17:20-17:35 - Maldi-tof MS fingerprinting of tarantula venoms: taxonomic and pharmacological applications. P. Escoubas, M. L. Célérier, C. Lucas, M. Rholam, T. Nakajima. (O-15Mo)

17:35-17:50 - All three free-swimming stages of *Linuche unguiculata* (Thimble jellyfish) can cause seabather' s eruption. L. Segura, J. Burnett, G. de Allende, C. Aramburo and E. Heimer de la Cotera. (O-16Mo)

17:50-18:05 - A comparison of australian and japanese redback spider venom. K.D. Winkel, C.J. Wiltshire, M. Yoshida, A. Kimura, Y. Okuno and S.K. Sutherland. (O-17Mo)

18:05-18:20 - Comparative studies on venom extracts from three jellyfish: the irukandji (*Carukia barnesi*), the box jellyfish (*Chironex fleckeri* Southcott) and the blubber (*Catostylus mosaicus*). C.J. Wiltshire, S.K. Sutherland, K.D. Winkel and P.J. Fenner. (O-18Mo)

18:20-18:35 - Domoic acid (ASP) implicated in marine animals casualties in the Gulf of California, México. A. P. Sierra-Beltrán, A.A. Cruz-V., S. Lluch-C, M. López-V., R. Rosiles, L. M. Del Villar-P., J. Cerecero and J.L. Ochoa. (O-19Mo)

18:35-18:50 - Toxicity of Japanese newt *Cynops pyrrhogaster* of western Kyushu region, Japan. Tamao Noguchi, Kazumi Tsuruda, Tomohiro Takatani, Hiroshi Akaeda and Osamu Arakawa. (O-20Mo)

18:50-19:00 - INTERVAL

D - SECOND PLENARY LECTURE

19:00-20:00 - **Dr. Findlay Russel:** "*Founding History of the International Society on Toxinology*" (PL-02Mo)

E - POSTER SECTION MOSTLY RELATED TO PURIFICATION, STRUCTURE, ELECTROPHYSIOLOGY AND FUNCTIONAL PROPERTIES

14:00-16:00 - Posters

P-001Mo: Mapping of the functional sites of a-conotoxins. Hung Lamthanh, Denis Servent and André Ménez

P-002Mo: Functional effects of BgK, a structurally new K^+ channel toxin on cloned *shaker* Kv 1.2 channels. C.L.C de Medeiros, M. Dauplais, C. Vita, A. Ménez, A.L. Harvey and E.G. Rowan

P-003Mo: A strategy for modeling the complex of CN2, a scorpion toxin from *Centruroides noxius* Hoffmann, with the neutralizing antibody BCF2. B. Selisko, E.S. Calderón, G. Gurrola, A.F. Licea, B. Becerril, L.D. Possani and E. Horjales

P-004Mo: Cobatoxins 1 and 2 from *Centruroides noxius* Hoffmann represent a new subfamily of K^+ channel blocking toxins. B. Selisko, C. García, F. Gómez, B. Becerril and L.D. Possani

P-005Mo: Transient K^+ -currents from cerebellum granular cells are completely blocked by a peptide purified from the scorpion *Androctonus australis* Garzoni venom. M. Pisciotta, F. Coronas, L. D. Possani, and G. Prestipino

P-006Mo: Amino acid sequences pharmacological properties, and molecular modeling of the two most potent ß-neurotoxins isolated from the venom of the Mexican scorpion *Centruroides suffusus suffusus*.F. sampieri, G. Bechis, P. Bougis, T. Brando, B. Céard, C. Granier, P. Mansuelle, M.F. Martin-Eauclaire and H. Rochat

P-007Mo: Structure-activity relationship studies of the toxin II from the scorpion *Androctonus australis hector*: modification of lysine and arginine residues. F. Sampieri, O. Hassani, R. Oughideni, C. Devaux, P. Mansuelle and H. Rochat.

P-008Mo: Epidemiology of the accidents caused by *Bothrops leucurus* in the metropolitan region of Bahia, Brazil (1982-1996). R.M. Lira-da-Silva and F.M. Carvalho.

P-009Mo: Three-dimensional crystal structure of muscarinic toxin 2 from *Dendroaspis angusticeps* venom. R. Ménez, M.H. Ledu and A. Ménez

P-010Mo: Analysis of secreted fluids from *Scolopendra suspinipes*. Jacques Pusset and Andrzej Galat

P-011Mo: Three dimensional structure of Calcicludine, an L-type calcium channel inhibitor extracted from the green mamba venom. Sophie Zinn-Justin, Alain Lecoq, François Desné, Bernard Gilquin and André Ménez

P-012Mo: Characterization of α-neurotoxin and phospholipase A_2 activities from *Micrurus* venoms: determination of amino acid sequence and receptor binding ability of the major α-neurotoxin from *Micrurus nigrocinctus nigrocinctus*. Jean-Pierre Rosso, Orietta Vargas-Rosso, José María Gutiérrez, Hervé Rochat and Pierre E. Bougis

P-013Mo: *Tityus caripitensis n. sp.* scorpion venom gland histology. M. Quiroga, M.J. de Marval, P. Parrilla-Alvarez and L. De Sousa

P-014Mo: Action of centipede *Scolopendra sp.* venom on the giant axon and on the cholinergic central aynaptic transmission of the cockroach *Periplaneta americana*. B. Hue, R. Benkhalifa, M. Stankiewicz, M.S. Lucas, R. Knysak, M. Pelhate, and D. Mebs

P-015Mo: Implication of the 8-10 region of an alpha type recombinant scorpion toxin in its electrophysiological properties. B. Bouhaouala-Zahar, R. Benkhalifa, F. Ducancel, I. Zenouaki, M. Pelhate, J. C. Boulain, M. El Ayeb, A. Menez, H. Karoui

P-016Mo: Lys-77 is important for hemolytic activity of equinatoxin II, a pore forming toxin from the sea anemone *Actinia equina*. G. Anderluh, A. Barlic, J. Pundercar, G. Menestrina, F. Gubensek and P. Macek

P-017Mo: Expression of intermediate filament proteins in mulgotoxin induced myodegeneration. D. Ponraj and P. Gopalakrishnakone

P-018Mo: Chibutoxin, a novel toxin isolated from the venom of the scorpion *Buthus martensi* Karsch which inhibits adrenergic transmission. JianPing Gong, M.C.E. Gwee, R. Manjunatha Kini and P. Gopalakrishnakone

P-019Mo: Capillary electrophoretic analysis of scorpion venoms. JianPing Gong, P. Gopalakrishnakone, R. Manjunatha Kini, and M.C. E. Gwee

P-020Mo: Polyamine spider toxins as a base for biospecific chromatography of insect muscle ionotropic glutamate receptor. P. V. Perestenko, M.J. Brierley, C.J. Kerry, I.V. Mosharova, T.M.

Volkova, P.N.R. Usherwood, E.V. Grishin

P-021Mo: Effects of pax isolated from the coral *Parazoanthus axinellae* on neuromuscular junction and on neuronal AChE. J. Strupi Suput, R. Frangrez, T. Turk and D. Suput

P-022Mo: Effects of a phospholipase A_2 fraction from *B. insularis* venom on the membrane resting potential and Ca^{2+} sensitivity of fast and slow muscle fibers. J.C. Cogo, D. Danieli-Betto, A. Megighian, A. Esposito, S. Parlatore, J. Prado-Franceschi, L. Rodrigues-Simioni and M. Midrio

P-023Mo: Purification and characterization of rat stomach contractile peptide from the venom of *Agkistrodon halys blomhoffii*. Y. Samejima, R. Yanoshita and A. Kasuga

P-024Mo: Ocurrence of paralytic sellfish poison in the starfish *Asterias amurensis* in Hiroshima Bay, Hiroshima prefecture, Japan. M. Asakawa, F. Nishimura, K. Miyazawa and T. Noguchi

P-025Mo: Determination of the cleavage site of the bait region of human α2-macroglobulin and rat murinoglobulin by a hemorrhagic metalloproteinase from *Bothrops jararaca* venom. M. Sugiki, K. Anai, E. Yoshida and M. Maruyama

P-026Mo: Neuronal acceptor for ammodytoxin in pig brain cortex. N. Vucemilo, F. Gubensek and I. Krizaj

P-027Mo: Protein engineering of the C-terminal half of ammodytoxin A. J. Pungercar, I. Krizaj, G. Anderluh, G. Ivanovski and F. Gubensek

P-028Mo: Characterization of muscarinic toxins MT1, MT2, MT3 and MT7.K. N. Bradley, E.G. Rowan, D. Jerusalinsky and A.L. Harvey

P-029Mo: Australian funnel-web spider toxins and α-scorpion toxins share a common binding site on cockroach and rat voltage-gated sodium channels. M.J. Little, G.M. Nicholson, C. Zappia, D. Gordon, M.F. Martin-Eauclaire and M.I. Tyler

P-030Mo: Modification of sticholysin II by free radicals. I.F. Pazos, C. Alvarez, V. Morera, D. Martínez, Y. García, M.E. Lanio and E. Lissi

P-031Mo: Anti-parasites effects of *Bothrops jararaca* venom. Adriana R. Gonçalves, Wanderley de Souza, Maurilio J. Soares and Elias W. Alves

P-032Mo: Hemolytic and caseinolytic activities of *Megalopyge sp.* (Lepidoptera- Megalopygiidae) bristles extract and cross-reactivity with antilonomic serum (Lepidoptera- Saturniidae).

A.C.M. Rocha Campos, R.M.G. Andrade, P.J. Spencer, C. Geldmacher, and W. Dias da Silva

P-033Mo: Lonomiatoxin: a 20kDa protein present in bristles extracts of *Lonomia obliqua* caterpillars extracts. A.C.M. Rocha Campos, E.W. Alves, P.D. Melo, O.L.T. Machado and W. Dias da Silva

P-034Mo: Metabolite profile of marine derived fungi. Joan Nielsen, Per Halfdan Nielsen, Jens Frisvad and Lone Gram

P-035Mo: Purification and partial characterization of a proteinase inhibitor from sea anemone *Condylactis gigantea* . M. Chávez, Sh. Gil, A. Fernández, V. Huerta, I. Pascual, L. Abreu, V. Morera, A. Saroyán, J. Delfin, G. Padrón, M. Cisneros, P. Joseph, J.L. Charli and J. Díaz

P-036Mo: Purification and partial characterization of a novel proteinase inhibitor from the sea anemone *Stichodactyla helianthus* . J. Díaz, V. Morera, J. Delfin, V. Huerta, G. Lima, M. Rodríguez de la Vega, B. García,, G. Padrón, I. Assfalg-Machleidt and M. Chávez

P-037Mo: Isolation of toxins from the Caribbean Sea anemones *Bunodosoma granulifera* and *Phyllactis floscuifera*. A. Aneiros, E. Karlsson, L. Beress, A. Garateix, J. Alvarez, E. Soto and E. Salinas.

P-038Mo: Amino acid sequence of the toxic spider peptide CSTX-2 and its structural and physiological differences to CSTX-1. W. Nentwig, L. Kuhn-Nentwig, J. Schaller and U. Kämpfer

P-039Mo: CSTX-4, a novel bactericidal and insecticidal peptide in the venom of *Cupiennius salei*. L. Kuhn-Nentwig, J. Schaller, B. Streb and U. Kämpfer

P-040Mo: *Bothrops jararaca* snakes produce several bothrojaracin isoforms following an individual pattern. R.Q. Monteiro, D.L.S. Dutra, C.R. Carlini, J.A. Guimaraes, C. Bon and R.B. Zingali

P-041Mo: Muscarinic selective toxins in *Dendroaspis viridis* venom. E. Kornisiuk, C. Cerveñansky, R. Durán, P. Alfaro, M. Alonso and D. Jerusalinsky

P-042Mo: Partial primary sequence and CD spectra study of a new neurotoxin from *Naja naja atra* venom. W.Y. Wang, Q.X. Meng, Q.M. Lu, N.K. Liu and Y.L. Xiong

P-043Mo: A fibrinogen-clotting enzime from *Trimeresurus stejnegeri* venom, and comparative study with other venom proteases. Y. Zhang, R. Gao, W.H. Lee, S. W. Zhu, Y.L. Xiong and

W.Y. Wang

P-044Mo: Seasonal variation of toxicity and responsable toxins in Taiwanese starfish *Astropecten scoparius*. S.J. Lin, Y.H. Tsai, and D.F. Hwang

P-045Mo: Isolation and characterization of seven potassium-channel peptide inhibitors from a Chinese scorpion venom. R. Romi-Lebrun, B. Lebrun, M.F. Martin-Euclaire, P. Escoubas, F.Q. Wu and T. Nakajima

P-046Mo: A single step purification method for basic myotoxins. P.J. Spencer, M. Boni-Mitake, H. Costa, S.D. Aird, N. Nascimento and J.R. Rogero

P-047Mo: New family of polypeptide toxins from ant venoms. L.V. Shevchenko, E.D. Nosyreva, D.Yu. Malkov, K.A. Pluzhnikov, E.V. Grishin

P-048Mo: Isolation of hemorrhagins from venoms in the family viperidae. Gloria R. Chapa, Celia García, María S. Ramírez, Luis R. Soto, Elda E.Sánchez, and John C. Pérez

P-049Mo: The isolation of metalloproteinases from the venom of the northern blacktail rattlesnake (*Crotalus molossus molossus*). Elda E. Sánchez and John C. Pérez.

P-050Mo: Seasonal variation of paralytic toxicity in Taiwanese crab *Lophozozymus pictor* and identification of the responsible toxin. D.F. Hwang and Y.H. Tsai

P-051Mo: The effect of *Pandinus imperator* scorpion venom on the K^+ channels of human peripheral blood lymphocytes. R. Gáspár, Jr.P. Mózes, Jr.Z. Varga, Gy. Panyi, S. Damjanovich and L.D. Possani

P-052Mo: Further characterization of proteins from the venom of the scorpion *Pandinus imperator*, that cause inhibition and activation of ryanodine binding to Ca^{2+} channels. G.B. Gurrola, F. Zamudio, R. Conde, B. Becerril, C. Arévalo, L.D. Possani and H.H. Valdivia

P-053Mo: Phopholipins, a new family of phospholipase A_2 present in the venom of scorpions. R. Condé, A. Torres-Larios, F.Z. Zamudio, F.I.V. Coronas, B. Becerril, L.D. Possani

P-054Mo: Isolation and characterization of a novel K^+-channel blocking toxin from the venom of the scorpion *Hadrurus aztecus*. A. Torres-Larios, T. Olamendi-Portugal, F. Gómez-Lagunas and L.D. Possani

P-055Mo: Haemorrhagin with fibrinolytic activity from Russell's viper venom. D.Chakrabarty, A. Gomes and D. Bhattacharyya

P-056Mo: Neutralization of edema, hemorrhage and myonecrosis induced by crotalid venoms. Jason Evans and Charlotte L. Ownby

II - CONTINUATION SPECIFIC SCIENTIFIC PROGRAM - (*divided into sections A to E*)

TUESDAY, 23TH SEPTEMBER 1997

A - THIRD PLENARY LECTURE

08:30-09:30 **Dr. Maria L. Garcia:** "*Scorpion toxins: tools for studying $K^{\pm}$ channels*", by M.L. Garcia, M. Hanner, H.G. Knaus and G. J. Kaczorowski. (PL-03Tu)

B - SYMPOSIUM NUMBER 2: "TOXINS AS POTENTIAL DRUGS"

Chairperson: Dr. André Ménez
Co-chairperson: Dr. George K. Chandy

09:35-10:05 - What can toxins tell us for drug discovery?, by Alan L. Harvey, K. N. Bradley, S. Cochran, E. G. Rowan, J.A. Pratt, J.A. Quillfeldt and D.A. Jerusalinsky. (S-08Tu)
10:05-10:35 - From noxiustoxin to *Shiva-3*, a peptide toxic to the sporogonic development of *Plasmodium berghei*, Lourival D. Possani, M. Zurita, F. Hernández, M. Delepierre and M.H. Rodríguez (S-09Tu)
10:35-10:55 - Refreshment Break
10:55-11:25 - Inhibition of mouse acetylcholinesterase by fasciculin: Crystal structure of the complex and mutagenesis of fasciculin, by Pascale Marchot, Y. Bourne, C.N. Prowse, J. Kanter, S. Camp, E. Ackerman, Z. Radic, P.E. Bougis and P. Taylor. (S-10Tu)
11:25-11:55 - Structure-activity relationship of insecticidal scorpion neurotoxins and their use for the design of novel insecticides, by Michael Gurevitz, O. Froy, N. Zilberberg, M. Turkov, E. Loret, D. Gordon and N. Chejanovsky. (S-11Tu)
11:55-12:25 - An N-type calcium channel blocker in the venom of *Conus magus* defines a new class of pharmaco-therapeutic agent, by Robert R. Luther, G. Miljanich and S. Bowersox. (S-12Tu)

12:25-14:00 - **LUNCH** (on own) - Editorial Board of Toxicon meeting during lunch - Room 6 - Salon Herradura)

14:00-16:00 - **Posters** (see below, letter E)

C - ORAL PRESENTATIONS

ROOM 1: *SUBJECT MOSTLY RELATED TO IMMUNOTHERAPY*
Chairperson: Dr. Julian White
Co-chairperson: Dr. José María Gutiérrez

16:00-16:15 - F(ab')2 snake antivenom (equine) use in Martinique: efficacy and side-effects. L. Thomas, B. Tyburn, J. Ketterlé, J. Lang, and the Research Group on snake bites in Martinique. (O-21Tu)

16:15:16:30 - Practical assessment of antivenoms. R.D.G. Theakston, P.G. Sells, G.D. Laing and A.M. Richards. (O-22Tu)

16:30-16:45 - A critical reappraisal of antivenom potency assays. M. Ismail, M. A. Abd-Elsalam, M.S. Al Ahaidib, N. Abdoon and A.J. Fatani. (O-23Tu)

16:45-17:00 - Neutralizing human anti-crotoxin scFV isolated from a non-inmunized phage library. D.F. Cardoso, P. Lafaye, F. Nato, V. Choumet, P. England, T. Vaughan, C. Bon, I. Mota and J.C. Mazie. (O-24Tu)

17:00-17-20 - Refreshment break

17:20-17:35 Polyspecific ovine Fab for the treatment of crotalid snakebite in the United States. R.C. Dart. (O-25Tu)

17:35:17:50 The simultaneous injection of native and toxoided bothropic venom abolishes the appearance of acute and chronic lesions induced by the native venom. I.F. Heneine, G.D. Cassali, O.A. Rocha and L.G.D. Heneine. (O-26Tu)

17:50-18:05 Purification, amino acid sequence, molecular cloning and expression of non-toxic protein from *Tityus serrulatus* scorpion that induces the production of antivenoms. C. Chávez-Olórtegui, S.C.G. Fonseca, A.M.M. Ferreira, A.P. Ferreira, M. Richardson, C.R. Diniz and E. Kalaphotakis. (O-27Tu)

18:05-18:20 Investigations on serotherapy and inmunoprotection against scorpion toxins: recombinant toxins approach. B. Bouhaouala-Zahar, F. Ducancel, I. Zenouaki, J. C. Boulain, M. El Ayeb, A. Ménez and H. Karoui. (O-28Tu)

18:20-18:35 Treatment and prevention of jellyfish envenomation. P.J. Fenner, J.A. Williamson, J.W. Burnett. (O-29Tu)

18:35-18:50 Elisa for the detection of toxins in accidental envenoming by *Androctonus australis garzonii*: correlation with clinical gradations. M.N. Krifi, K. Dellagi and M. El Ayeb (O-30Tu)

18:50-19:00 INTERVAL
19:00-20:00 FOURTH PLENARY LECTURE (See below, letter D)

ROOM 2: *SUBJECT MOSTLY RELATED TO ION-CHANNELS AND INVERTEBRATE TOXINS*

Chairperson: Dr. Alan Harvey
Co-chairperson: Dr. Marcel Pelhate

16:00-16:15 - Voltage clamp studies of *Bunodosoma granulifera* toxin on the outward membrane currents in snail neurons. E. Soto, A. Garateix, R. Vega, J. Cebada and A. Aneiros. (O-31Tu)

16:15:16:30 - Are the voltage-dependent sodium channels the only one target for alpha scorpions toxins? M. Stankiewicz, B. Lapied, E. Zlotkin, H. Rochat and M. Pelhate. (O-32Tu)

16:30-16:45 - Release of acetylcholine induced by toxin Ts-γ is due to stimulation of Na^+ channels. Y.A. Drumond, T. Moraes-Santos, J.S. Cruz, P.S.L. Beirao and L. Freire-Maia. (O-33Tu)

16:45-17:00 - Activation of the calcium-dependent chloride current from *Xenopus* oocytes by maitoxin. L. Escobar, M. Martínez, C. Salvador, V. Morales and L. Vaca. (O-34Tu)

17:00-17-20 - Refreshment break

17:20-17:35 Genomic organization of scorpion toxins active on K^+ channels and evidence for a new class of toxins. C. Legros, B. Céard. P.E. Bougis and M.F. Martin-Eauclaire. (O-35Tu)

17:35:17:50 Possible mechanism/s of skeletal muscle degeneration by myoglobinuric-general myotoxins.P. Gopalakrishnakone and D. Ponraj. (O-36Tu)

17:50-18:05 Differential actions of Pacific ciguatoxin-1 on voltage-gated sodium channels of mammalian sensory neurons: reversal by D-mannitol. L.C. Birinyi, G.M. Nicholson and R. J. Lewis. (O-37Tu)

18:05-18:20 Equinatoxin II increases intracellular calcium and cell volume in NG108-15 cells and in smooth muscle cells. D. Suput, R. Frangez, F. Meunier and J. Molgo. (O-38Tu)

18:20-18:35 Comparison and characterization of the venoms of three *Parabuthus* scorpion species occurring in Southern Africa. J. Tytgat, T. Debont, A. Swerts, J. J. van der Walt, G. J. Müller, F. Verdonck and P. Daenens. (O-39Tu)
18:35-19:00 - INTERVAL

D. - FOURTH PLENARY LECTURE

19:00-20:00 - **Dr. Michel Lazdunsky:** "*A family of receptor for venom phospholipase* A_2", by G. Lambeau, P. Ancian, J. Barhanin, S. Beiboer, J.P. Nicolas, H.Veheij, E. Zwaritch, Y. Lin, M. Gelb and M. Lazdunski. (PL04-Tu)

E - POSTER SECTION ON IMMUNOTHERAPY AND CLONING

14:00-16:00 Posters
P-057Tu: Nucleotide sequence and expression of the gene coding for Helothermine, a toxin from *Heloderma horridum horridum*. L. Pardo, B. Becerril and L.D. Possani

P-058Tu: Encapsulation of crotoxin into liposomes and its association with inmunostimulants for the production of antivenom against *Crotalus durissus terrificus* snake. T.V. Freitas and F. Frézard

P-059Tu: Effect of detoxification caused by the incorporation of *Bothrops jararaca* venom into dehydration rehydration vesicles and evaluation of liposome stability. V.T. Carvalho and T.V. Freitas.

P-060Tu: Detoxification with formaldehyde of Venezuelan *Tityus* genus scorpion venoms. Inmunogenicity of toxoid as compared to crude venom. P. Parrilla-Alvarez, G. D' Suze, M. Quiroga, A. Rodriguez-Acosta, and C. Sevcik.

P-061Tu: Importance of alpha-macroglobulins in the control of hemorrhagic metalloproteinase envenomation. M. Maruyama, Y. Kikuchi, K. Anai, M. Sugiki and E. Yoshida.

P-062Tu: Neutralizing capacity of *Gallus domesticus* serum against Russell' s viper venom. M.I. Alam and A. Gomes.

P-063Tu: Antitoxin to *holocyclotoxin*, the paralysing toxin of the Australian paralysis tick, *Ixodes holocyclus* and its evaluation. B.F. Stone.

P-064Tu: ELISA for the detection of venoms in experimental and clinical envenoming by *Loxoceles intermedia* spiders. V. Zanetti, A. Braz, J. Minozzo, I. Gubert and C. Chávez-Olórtegui.

P-065Tu: Preparation of a transudate from *Bothrops jararaca*, using a surgical implant, that contains antihemorrhagic activity against the autologous venom. R.H. Valente, P.G. Sá, P.L. Cavalcanti, G.B. Domont and J. Perales.

P-066Tu: New methodology for the production of the antibothropic complex from *Didelphis marsupialis* (opossum). A.G.C. Neves-Ferreira, P.G. Sá, N. Cardinale, G.B. Domont, H. Moussatche and J. Perales.

P-067Tu: Characterization of the 48 kDa subunit from the antibothropic complex isolated from *Didelphis marsupialis* (opossum) serum. N. Cardinale, A.G.C. Neves-Ferreira, G.B. Domont, H. Moussatché, and J. Perales.

P-068Tu: Production in *E. coli* of scorpion toxins from *Androctonus australis* scorpion venom. C. Legros, S. Breyer, B. Céard, P.E. Bougis and M.F. Martin-Eauclaire

P-069Tu: Usefulness of a non-toxic analog to elict neutralizing poly- and mono-clonal antibodies against scorpion toxin. C. Devaux, O. Clot-Faybesse, M. Juin, K. Mabrouk, J.M. Sabatier, M. El Ayeb and H. Rochat.

P-070Tu: Inmunological cross reactivity among venoms of *Trimeresurus* species in Thailand and Japan. N. Pakmanee, O. Khow, N. Wongtongkam, T. Omori-Satoh, W. Kumsap, S. Sriprapat and V. Sitprija.

P-071Tu: Development of reversed passive latex agglutination for detection of Thai cobra (*Naja kaouthia*) venom. O. Khow, N. Wongtongkam, N. Pakmanee, T. Omori-Satoh and V. Sitprija.

P-072Tu: Comparative study of bothrojaracin, with homologous proteins. H.C. Castro, M.G. Lemos and R.B. Zingali.

P-073Tu: A field trial with $(ab')_2$ antivenom in North Cameroon. J.P. Chippaux, S. Amadi-Eddine and P. Fagot.

P-074Tu: Treatment of snakebite by trypsin and chymotrypsin. Y. L. Xiong, W.Y. Wang, Y. Zhang, W.H. Lee, T.R. Xu, Q.X. Meng, Q. M. Lu, S.W. Zhu and D.S. Li.

P-075Tu: Studies on some protective manoeuvres in mulgotoxin induced rhabdomyolysis and renal damage. Ponraj D. and Gopalakrishnakone P.

P-076Tu: Human high molecular weight kininogen inhibits hemorrhagic activity of *Bothrops jararaca* (Bj) venom. L.R.C. Gonçalves and A.M. Chudzinski-Tavassi.

P-077Tu: Characterization of *Micrurus frontalis altirostris* venom. Francisco Valls-Moraes and Maria de Fátima D. Furtado.

P-078Tu: Serum levels of cytokine in patients envenomed by *Tityus serrulatus* scorpion sting. M. M. Magalhaes, M. E. S. Pereira, C.F.S. Amaral, N.A. Rezende, D. Campolina, M.B. Dias, F. Bucaretchi, R.T. Gazzinelli and J. R. Cunha-Melo.

P-079Tu: Tourniquet ineffectiveness to reduce the severity of envenoming after *Crotalus durissus* snake bite in Monas Gerais, Brazil. C.F.S. Amaral, N.A. Rezende, M.B. Dias, C.M. Bueno, R.L. Martins, P.M.B. Fillho, F.M.T. Alves and D. Campolina.

P-080Tu: The development of the five step western blot. Tammy L. Bires, Sandra De La Zerda, and John C. Perez.

P-081Tu: Improved ELISA for Russell's viper (*Daboia russelii*) antivenom potency. Jarasporn Rungsiwongse and Kavi Ratanabanangkoon.

P-082Tu: Inhibition by $CaNa_2EDTA$ of local tissue damage induced by *Bothrops asper* venom: application in horse inmunization for antivenom production. J. M. Gutiérrez, G. León, R. Estrada, F. Chaves, G. Rojas and M. Ovadia.

P-083Tu: Development of an ELISA to assess the potency of horse therapeutic polyvalent antibothropic antivenom. L.G.D. Heneine, A.D. Carvalho Júnior and M.R.A. dos Santos.

P-084Tu: Tissue and serum kinetcs of *Tityus serrulatus* scorpion antivenom: An experimental study in mice. M.P. Revelo, E.A. Bambirra, A.P. Ferreira, C.R. Diniz, C. Chávez-Olórtegui.

P-085Tu: Neutralizing ability of a new monovalent anti-*Loxosceles intermedia* antivenom. Comparison with anti-arachnidic antivenom. A. Braz, J.C. Minozzo, M. Cabuí, I.C. Gubert and C. Chávez-Olórtegui.

P-086Tu: Potency of horse antivenoms against *Bothrops sp* venom. Correlation between *in vitro* and *in vivo* assays. W.S. Maria, D. T. Velarde, J.O. Costa and C. Chávez-Olórtegui.

P-087Tu: Use of different inmunogens from production of horse anti-scorpionic antivenom. D.T. Velarde, W.S. Maria, A.P. Ferreira and C. Chávez-Olórtegui.

P-088Tu: Antivenenum and enzyme-inmuno-assay for cobra venom poisoning treatment. T.X. Kiem, T.K. Anh, L.K. Quyen, N.B. Phuoc, L.T.N. Thu and N.L. Trang.

P-089Tu: Effects of anticonvulsant drugs on the experimental severe scorpionism elicited by *Tityus serrulatus* venom in mice. C. Souza, V.S. Fernandes and M.B. Farah.

P-090Tu: Phenytoin protects against the lethality induced by the *Tityus serrulatus* scorpion venom in mice. M.B. Farah, C. Souza, and V.S. Fernandes.

P-091Tu: TH1 and TH2 functions is down regulated by *Crotalus durissus terrificus* venom. D.F.Cardoso,, E. Faquim-Mauro, M.S. Macedo, M. Colombini, F. Nato, P. Lafaye, J.C. Mazié, and I. Mota.

P-092Tu: Development of snake antivenom in chicken and its recovery from the yolk as therapeutic tool for domestic animals. C.M.C. Almeida, M.M. Kanashiro, F.B. Rangel Filho M.F.R. Matta, T.L. Kipnis and W. Dias da Silva.

P-093Tu: Kinetic analysis of pore formation by sticholysin I. M. Tejuca, M. Dalla Serra, M. Ferreras, C. Alvarez, M.E. Lanio and G. Menestrina.

P-094Tu: Production and characterization of neutralizing antibody against a thrombin-simile purified from *Bothrops atrox* venom. M.M. Kanashiro, J.H. Petretski, E.W. Alves, T.L. Kipnis.

P-095Tu: Design, synthesis and functional expression of recombinant Pi1, a K^+-channel blocker. F. Martínez, F. Gómez-Lagunas, T. Olamendi-Portugal, B. Martin and L.D. Possani.

P-096Tu: Epitope mapping for monoclonal antibodies against LTX. V.D. Lazareva, K.E. Volynsky, N.E. Bocharova, T.M. Volkova, V.N. Pashkov, E.V. Grishin.

P-097Tu: OsK1 cDNA Cloning and expression in *E. coli*. Yu V. Korolkova, A.V. Lipkin. K.A. Pluzhnikov and E.V. Grishin.

P-098Tu: Functional expression of δ-latroinsectotoxin in *E. coli* cells. K.E. Volynsky, M.V. Khvochtchev, N.E. Bocharova and E.V. Grishin.

P-099Tu: Cloning of genes encoding isoforms of α-neurotoxins in *Naja naja sputatrix* venom. F. Afifiyan, A. Armugam, N.H. Tan, C. H. Tan and K. Jeyaseelan.

P-100Tu: Genes encoding isoforms of cardiotoxin in *Naja naja sputatrix*. R. Lachumanan, A. Armugam, P. Gopalakrishnakone, C. H. Tan and K. Jeyaseelan.

P-101Tu: Genomic organization of a K^+-channel toxin gene from sea anemone: a common path of the evolution among ion-channel toxin genes? Gurmil S. Gendeh, Max C. M. Chung and Kandiah Jeyaseelan.

P-102Tu: Cloning and sequence analysis of a *Bothrops jararaca* cDNA encoding a precursor of seven bradykinin-potentiating peptides and a C-type natriuretic peptide. N. Murayama, M.A.F. Hayashi, H. Ohi, L.A.F. Ferreira, V.V. Hermann, H. Saito, Y Fujita, S. Higuchi, B.L. Fernandes, T. Yamane and A.C.M. Camargo.

P-103Tu: Cloning and sequence analysis of serine protease cDNAs from *Bothrops jararaca*. Y. Hagiwara, N. Murayama, K. Saguchi, H. Ohi, Y. Fujita, S.M.T. Serrano, A.C.M. Camargo and S. Higuchi.

P-104Tu: Cloning, expression and sequence analysis of a new metalloproteinase/disintegrin from *Agkistrodon contortrix laticinctus*. H.S.Selistre-de-Araujo, D.H.F. Souza, and C.L. Ownby.

P-105Tu: Presence of myotoxin *a* mRNA in venom gland and liver

samples from prairie rattlesnake (*Crotalus viridis viridis*). E. Knight, T.R. Colberg and C.L. Ownby.

P-106Tu: Cloning and sequence analysis of genes encoding phospholipase A_2 in the venom of *Naja naja sputatrix*. A. Armugam, S.L. Kok, R. M. Kini, P. Gopalakrishnakone, N. H. Tan and K. Jeyaseelan.

P-107Tu: Long and weak neurotoxins from *Naja naja sputatrix* : cDNA cloning and sequence analysis. S.L. Poh, A. Armugam and K. Jeyaseelan.

P-108Tu: Crotalinae snake venom gland serine proteases have evolved *via* Darwinian-type accelerated evolution. M. Deshimaru, T. Ogawa, K. Nakashima, I. Nobuhisa, T. Chijiwa, Y. Shimohigashi, Y. Fukumaki, S. Hattori and M. Ohno.

P-109Tu: Structure of group I snake venom phospholipase A_2 gene from *Laticauda semifasciata*. T.J. Fujimi, S. Ohno, Y. Tabara, T. Tamiya and T. Tsuchiya.

P-110Tu: Classification of sea snakes in genus *Laticauda* by nucleotide sequences encoding short chain neurotoxins. Y. Kariya, S. Araki, H. Agu, T. Tamiya and T. Tsuchiya.

II - CONTINUATION SPECIFIC SCIENTIFIC AND SOCIAL PROGRAM -
(divided into sections A to D)

WEDNESDAY, 24TH SEPTEMBER 1997

A - SYMPOSIUM NUMBER 3: "FOOD POISONING"

Chairperson: Dr. Dietrich Mebs
Co-chairperson: Dr. Gerhard Habermehl

08:30-09:00 - Fish poisoning due to toxins of microalgal origins in the Pacific, by Takeshi Yasumoto. (S-13We)
09:00-09:30 - Cytotoxic and ichthyotoxic compounds from marine opisthobranchia and soft corals, by Ryuichi Higuchi (S-14We)
09:30-10:00 - Ciguatoxins on serranidae and lutjanidae fish of Baja California Sur, Mexico, by E.J. Nuñez-Vázquez, Arturo P. Sierra-Beltrán, A.A. Cruz-Villacorta and J.L. Ochoa. (S-15We)
10:00-10:30 - Ocurrence, impact and management of cyanobacterial blooms and toxins in European freshwater and marine environments, by Geoffrey A. Codd. (S-16We)
10:30-11:00 - Ocurrence and sequestration of toxins in food chains, by Dietrich Mebs. (S-17We)

11:00-11:15 - Refreshment break

B - FIFTH PLENARY LECTURE

11:15-12:15 *REDI AWARD LECTURE (PERSON TO BE NOMINATED)*

12:15-20:00 - **BOX-LUNCH FOR TRIPS, VISITS OR JUST RELAX**

C - 20:00 - DINNER OF THE CONGRESS

D - 21:00-22:20 - FOLKLORIC BALLET OF THE STATE OF MORELOS
"*COMPAÑIA DE DANZA FOLKLORICA TLAHUICA*"

II - CONTINUATION SPECIFIC SCIENTIFIC PROGRAM - (*divided into sections A to E*)

THURSDAY, 25th SEPTEMBER, 1997

A - SIXTH PLENARY LECTURE

08:30-09:30 **Dr. Eugene Grishin:** "*Black widow spider toxins: the present and the future*" (PL-05Th)

B - SYMPOSIUM NUMBER 4: "IMMUNOTHERAPY"

Chairperson: Dr. Cassian Bon
Co-chairperson: Dr. David Theakston

09:35-10:00 - The development and the use of the immunotherapy in Africa, by Jean-Philippe Chippaux (S-18Th)
10:00-10:25 - Envenoming and antivenom use in Australia, by Julian White. (S-19Th)
10:25-10:50 - Antivenom neutralization of local tissue damage induced by snake venoms, by José María Gutiérrez, B. Lomonte, G. Léon and G. Rojas. (S-20Th)
10:50-11:10 - Refreschment Break
11:10-11:35 - New approaches to optimize antivenom therapy, by Valérie Choumet, G. Rivière, F. Audebert, M. Sorkine and C. Bon. (S-21Th)
11:35-12:00 - Immunotherapy for scorpion envenoming in Brazil, by Lineu Freire-Maia, C.F.S. Amaral and N.A. Rezende. (S-22Th)
12:00-12:25 - Pharmacokinetics of ^{125}I-Labelled IgG, F(ab')$_2$ and Fab fractions of scorpion and snake antivenins: merits and potential for therapeutic use, by Mohammad Ismail and M.A. Abd-Elsalam. (S-23Th)

12:25-14:45 - **LUNCH WITH POSTER SECTION ON BIOASSAYS, BIOLOGICAL EFFECTS, MICROBIAL TOXINS AND OTHERS** (See list in the detailed scientific program, letter E below)

C - SYMPOSIUM NUMBER 5: "STRUCTURE AND FUNCTION OF SEVERAL TOXINS"

Chairperson: Dr. Peter Strong
Co-chairperson: Dr. Hervé Rochat

14:45-15:15- Mapping the pore of the T-lymphocyte potassium channel with scorpion and sea-anemone toxins: from toxin to immunosuppressant, by George K. Chandy (S-24Th)

15:15-15:45- ^{1}H-NMR structural analysis, using a nano-NMR probe, of a novel potassium channel blocking toxin from the scorpion *Pandinus imperator*, by Muriel Delepierre, A. Prochnicka-Chalufour and L.D. Possani. (S-25Th)

15:45-16:15- Ion channel toxins: 3D structures, channel-binding surfaces and progress towards minimisation, by Raymon S. Norton. (S-26Th)

16:15-16:45- Maurotoxin, a four disulfide bridges scorpion toxin acting on K^+ channels, by Hervé Rochat, R. Kharrat, J.M. Sabatier, P. Mansuelle, M. Crest, M. F. Martin-Euclaire, F. Sampieri, R. Oughideni, K. Mabrouk, G. Jacquet, J. Van Rietschoten and M. El Ayeb. (S-27Th)

16:45-17:15- Identification of potential functional sites in proteins: toxins to therapeutics, by R. Manjunatha Kini. (S-28Th)

17:15-17:30 - Refreshment Break

D - THREE SIMULTANEOUS ORAL PRESENTATIONS: BIOLOGICAL EFFECTS, INTERACTION OF VENOM COMPONENTS WITH THEIR RECEPTORS AND IMMUNOTHERAPY II

ROOM 1: *SUBJECT MOSTLY RELATED TO BIOLOGICAL EFFECTS*
Chairperson: Dr. Carlos Sevcik
Co-chairperson: Dr. Júlia Prado-Franceschi

17:30-18:00 - Shaul Sofer will present three titles:
1. Effects of scorpion venom on central and peripheral circulatory response in opened-chest dog model. S. Sofer and A. Tarasiuk. (O-40Th)
2. Cardiovascular effects in dogs following injection of venom from the *Leiurus quinquestriatus* scorpion. S. Sofer, S. M. Scharf and A. Tarasiuk. (O-41Th)
3. Scorpion venom leads to gastointestinal ischemia in pigs despite increased oxygen delivery. S. Sofer, R. Cohen, Y. Shapir, L. Chen, A. Colon and S. M. Scharf. (O-42Th)

18:00-18:15: Lack of effect of *Tityus discrepans* scorpion venom on isolated rabbit lung. G. D'Suze, A. Comellas, C. Sevcik and R. Sánchez de Léon. (O-43Th)

18:15-18:30: Neutralization of the myotoxic and neurotoxic activities of *Crotalus durissus terrificus* venom and of its principal component, crotoxin. Y. O. Franco, L. Rodrigues-Simioni and J. Prado-Franceschi. (O-44Th)

18:30-18:45: Physiological basis of neuromuscular paralysis caused by toxic phospholipases A_2. R. Dixon and J. B. Harris. (O-45Th)

18:45-19:00: Reorganisation of motor innervation of skeletal

muscle exposed in vivo to ß-bungarotoxin. Celia Burgoyne, Steve Walker, Kristina Warzynska, Rupert Dixon and John Harris.(O-46Th)

19:15-19:30: Palytoxin and transmitter release in the skeletal neuromuscular system. K. Warzynska, A. Gibson, J. Court, D. Mantle and J. Harris. (O-47Th)

19:30-19:45: Blood platelet activation by venom of African green bush viper (*Atheris squamiger*). F. Kornalík, D. Mebs, J. Simak, K. Holada, H. Zd'árská, H.-W. Herrmann, Z. Kucerová, H. Vanková. (O-48Th)

19:45-20:00: How does honey bee venom kill? Justin O. Schmidt (O-49Th)

20:00-20-15 Isolation and partial amino acid sequence of a novel metalloprotease from the venom of the colubrid snake Hydrodynastes gigas (false water cobra). Stephen P. Mackessy. (O-50Th)

ROOM 2: *SUBJECT MOSTLY RELATED TO INTERACTION OF VENOM COMPONENTS WITH THEIR RECEPTORS*

Chairperson: Dr. Michel Gurevitz
Co-chairperson: Dr. R. Manjunatha Kini

17:30-17:45 - A new SK_{Ca} channel toxin from *Oxyuranus scutellatus* taipan venom. K.B. Doorty, S. Bevan, J.D.F. Wadsworth and P. Strong. (O-51Th)

17:45-18:00: Epitope mapping of the nicotinic acetylcholine receptor using photoaffinity derivatives of α-neurotoxins from cobra venom. Patricio Sáez-Briones, Ferdinand Hucho and Victor Tsetlin. (O-52Th)

18:00-18:15: Specific neurotoxins interaction on muscular and neuronal (α7) nicotinic acetylcholine receptor. D. Servent, V. Winckler-Dietrich, Hai-Yan Hu, P. Kessler, P. Drevent, D. Bertrand and A. Ménez. (O-53Th)

18:15-18:30: Natural ligands with selectivity for subtypes of muscarinic receptors. D. Jerusalinsky, E. Kornisiuk, K. N. Bradley, A. L. Harvey, P. Alfaro, M. Alonso, R. Durán and C. Cerveñansky. (O-54Th)

18:30-18:45: Decreased collagen-stimulated phosphorylation of the platelet tyrosine kinase pp^{72syk} by the venom metalloproteinase jararhagin. A. S. Kamiguti, F.S. Markland, Q. Zhou, G.D. Laing, R.D.G. Theakston and M. Zuzel. (O-55Th)

18:45-19:00: SRTX-d/e inhibits sarafotoxin-b-induced contraction of guines pig intestine. Z. Wollberg, A. Matz, S. Kinamon and A. Bdolah. (O-56Th)

19:15-19:30: A peptide ligand for glutamate receptors from the venom of the tarantula *Phlogiellus sp.* P. Escoubas, D. Mebs, K. Shimamoto, T. Nakajima. (O-57Th)

19:30-19:45: Interaction of TX1, a neurotoxin from the spider *Phoneutria nigriventer* venom, with the guinea pig ileum. R. Gouvea dos Santos, C.R. Diniz, M.N. Cordeiro and M.E. De Lima. (O-58th)

19:45-20:00: A novel four disulfide-bridged scorpion toxin, with high affinity towards voltage-gated K^+ channels. B. Lebrun, R. Romi-Lebrun, M. F. Martin-Euclaire, O. Pongs and T. Nakajima. (O-59Th)

20:00-20:15: Fibrin glue derived from snake venom: use in dermatological surgery. H.O. Stolf, S.R.C.S. Barraviera, I.A. Thomazini-Santos, E. Toscano, M.J.S. Mendes-Giannini and B. Barraviera. (O-60Th)

ROOM 3: *SUBJECT MOSTLY RELATED TO IMMUNOTHERAPY - II*
Chairperson: Dr. Carlos Diniz
Co-chairperson: Dr. Bruno Lomonte

17:30-17:45 - Molecular cloning and sequencing of the variable region genes of the heavy and light chains of human anti-tetanus monoclonal antibody MAB-G6 with high neutralizing activity. M. Matsuda, J. Katahira, M. Kamei, S. Hashizume and N. Sugimoto. (O-61Th)

17:45-18:00: Targeting to immune cells increases toxin immunogenicity. Michel Léonetti and André Ménez.(O-62Th)

18:00-18:15: Efficacy of two dosing regimens of affinity purified, mixed monospecific crotalid antivenom ovine FAB (crotab). G. M. Bogdan, S. Seifert, L. Boyer, R. Clark, E. Hall, P. McKinney, C. Kitchens, S. Curry, D. Smith, R.S. Porter, R.C. Dart. (O-63Th)

18:15-18:30: *Bothrops asper* myotoxin inhibitor protein (BaMIP): a novel anti-toxic and anti-phospholipase A_2 factor from the blood of *B. asper*. S. Lizano, B. Lomonte, and J.M. Gutiérrez. (O-64Th)

18:30-18:45: Envenomation by *Tityus stigmurus* in the metropolitan region of Bahia, Brazil. R. M. Lira-da-Silva, A. M. Amorim and T.K. Brazil. (O-65Th)

18:45-19:00: Horse IgG isotypes present in antivenoms produced by institutes Clodomiro Picado (CR) and Butantan (BR). I. Fernandes, H.A. Takehara, E.X. Lima, J.M. Gutierrez and I. Mota. (O-66Th)

19:15-19:30: *Thalassophryne nattereri* fish venom: biochemical characterization and neutralization of toxic activities. M. Lopes-Ferreira, K.C. Barbaro, D.F. Cardoso, A.M. Moura-da-Silva and I. Mota. (O-67Th)

19:30-19:45: Cobra antivenom evaluation anticobra antiserum produced in Vietnam. T.X. Kiem, T.K. Anh, L.K. Quyen, N.B. Phuoc, N.L. Trang, N.T.N. Thu. (O-68Th)

19:45-20:00: A quantitative comparison on the refinement of horse antivenom by salt fractionation and ion-exchange chromatography. T. Saetang, N. Tremwattana, P. Suttijitpaisal and K. Ratanabanangkoon. (O-69Th)

20:00-20:15: Compared pharmacokinetic and efficacy of $F(ab')_2$ and Fab in experimental viper envenomations. G. Rivière, V. Choumet, B. Saliou and C. Bon. (O-70Th)

E - POSTER SECTION ON BIOASSAYS, BIOLOGICAL EFFECTS, MICROBIAL TOXINS AND OTHERS

12:25-14:25 Lunch and Poster together (by the Congress)

P-111Th: Neutrophil migration induced in mouse by the staphylococcal enterotoxin type A: a pharmacological analysis. I.A. Desouza and G. Ribeiro-DaSilva.

P-112Th: Structural organization of the different domains from Cry1Ab toxin of *Bacillus thuringiensis* in the membrane inserted state. Raúl Miranda, Gloria Guerrero and Alejandra Bravo.

P-113Th: Isolation and characterization of peptides from a phage variant library which are able of binding to Cry1A(b) δ-endotoxin as its natural receptor. M. Peralta, A. Bravo and M. Soberón.

P-114Th: Is bacterial adherence through CFA-I a necessary step for secretion of the periplasmic heat labile toxin in enterotoxigenic *Escherichia coli* ? L. Y. Fuchs, B. Carrillo, L. Gutierrez, H. Valdovinos, F. Cassels, A. Cravioto, Y. M. Kupersztoch.

P-115Th: Differences in mode of action of domain I from *Cry* 4A and *Cry* 11A toxins from *Bacillus thuringiensis* in mosquito midgut membranes. S. Tenorio, J. Sanchez and A. Bravo.

P-116Th: Is aminopeptidase N the receptor of *Cry* 1 Ac δ-endotoxin in *Trichoplusia ni* midgut? A. Lorence, A. Darszon and A. Bravo.

P-117Th: The insecticidal crystal protein family from *Bacillus thuringiensis*. A. Bravo, A. Lorence, J. Sánchez, H. Flores, L. Güereca and M. E. Nuñez.

P-118Th: Chemical conversions of the tumor promoting mycotoxin fumonisin B1 from *Fusarium moniliforme* during food processing W.T. Shier, P.A. Tiefel and F.A. Badria

P-119Th: Characterization of a lethal and paralysing toxin (acyl polyamine) isolated from the venom of *Scaptocosa raptoria* spider. L.A.F. Ferreira, M.S. Lucas, M.B. Riverón, A.P. Reichl, Q.M. Silva, B. Fernandes and G. Habermehl.

P-120Th: Clinical features of envenoming by *Bothrops leucurus* snakebite in metropolitan region of Salvador, Bahía, Brazil. R. M. Lira-da-Silva and F. M. Carvalho.

P-121Th: Increase of vascular permeability in rat induced by *Micrurus lemniscatus* venom. L.L. Casais e Silva and C.F.P. Teixeira.

P-122Th: Recruitment of neutrophils and release of inflammatory mediators induced by *Tityus serrulatus* venom (TsV) in the lung of rats. C.F.P. Teixeira, F. Galante, A.A. Steil, N. Starobinas and S. Jancar.

P-123Th: Functional alterations induced in peritoneal macrophages by myotoxins isolated from *Bothrops asper* venom (BaV). F. Galante, L.L. Casais e Silva, M.C. Toffoli, Y. Cury, J.M. Gutiérrez and C.F.P. Teixeira.

P-124Th: Hyperalgesia induced by *Bothrops asper* venom (BaV) and myotoxin III in rats. G. Picolo, J.M. Gutierrez, C.F.P. Teixeira and Y. Cury.

P-125Th: Effect of *Bothrops asper* venom (BaV) on leukocyte-endothelial interactions. A direct vital microscopic study. S. H. P. Farsky, L. R. C. Gonçalves and J.M. Gutierrez.

P-126Th: Characterization of local tissue damage evoked by *Bothrops jararaca* venom (BjV) in the rat connective micro-circulation. An intravital microscopic study. S.H.P. Farsky, L.R. C. Gonçalves and Y. Cury.

P-127Th: Myotoxic activity of an acidic PLA_2 from *Lachesis muta* snake venom. A.L. Fuly, P.A. Melo, C.R. Carlini and J.A. Guimaraes.

P-128Th: The study of analgesic effect of neurotoxins from *Naja naja atra* venom. Y.L. Xiong, W.Y. Wang, W.H. Lee, Q.X. Meng, Q.M. Lu, S. W. Zhu, D.S. Lee, W.G. Sun, R.H.Zhang, H. Zhu and H.

Huang.

P-129Th: Characterization of a prothrombin activator from *Agkistrodon halys pallas* venom. Y. Zhang, W.H. Lee, R. Gao, Y.L. Xiong, W.Y. Wang and S.W. Zhu.

P-130Th: Mechanism of the effect of a phospholipase A_2 platelet inhibitor from *Ophiophagus hannah* venom. M. Z. Huang, P. Gopalakrishnakone and R. Manjunatha Kini.

P-131Th: The action of *Bothrops neuwiedi* venom on chick *Biventer cervicis* and mouse phrenic nerve-diaphragm preparations. C.R. Borja, S. Hyslop and L. Rodrigues-Simioni.

P-132Th: Envenomation of children by snakes of the genera *Bothrops* and *Crotalus*. F. Bucaretchi, E.C.E. Baracat, M.T. Nolasco, M.R.C.C. Fonseca, F. Tourinho Pereira and R.J. Vieira.

P-133Th: Local effects of *Bothrops lanceolatus* venom. A. Lobo de Araújo, L. de Faria, and C. Bon.

P-134Th: Characterization of the nociceptive and edematogenic activities of the *Thalassophryne nattereri* fish venom. M. Lopes-Ferreira, J.A.S.Emim, C. Souccar, A.J. Lapa, M.H.S. Cezari, L. Juliano, A.M. Moura-da-Silva and I. Mota.

P-135Th: Inflamatory response induced by *Micrurus frontalis altirostris* venom. M.C.C. Sousa-e-Silva, F. Valls-Moraes, M. Leitao de Araujo and M.L.M. Alves.

P-136Th: Evidence that antinociception induced by *Crotalus durissus terrificus* venom on inflammatory hyperalgesia is partialy mediated by peripheral opioid receptors. G. Picolo, R. Giorgi and Y. Cury.

P-137Th: *Crotalus durissus* snakebite revisited. C.F.S. Amaral, D. Campolina, M.B. Dias, C.M. Bueno, G.L. Adjuto, R.M.C. Monteiro, W.K. De Paula and N.A. Rezende.

P-138Th: Effects of the metalloproteinases, mutalysin I and II on the human blood coagulation and fibrinolysis systems. M.I.E. Costa, C.R. Diniz and E.F. Sánchez.

P-139Th: Snake bite mortality in Costa Rica. G. Rojas, G. Bogarín and J.M. Gutiérrez.

P-140Th: Envenomation of mice by Thai cobra (*Naja kaouthia*) venom: tolerable venom concentration and exposure time. N. Pakmanee, O. Khow, W. Kumsap, T. Omori-Satoh, L. Chanhome, S. Sriprapat and V. Sitprija.

P-141Th: Comparative study of the cytolytic activity of myotoxic phospholipases A_2 on murine endothelial and skeletal muscle cells *in vitro*. B. Lomonte, Y. Angulo, S. Rufini, W. Cho, J.R. Giglio, M. Ohno, J.J. Daniele, P. Geoghegan, and J.M. Gutiérrez.

P-142Th: Lysine-49 phospholipase A_2 and its synthetic peptide 115-129 kill bacteria. L. Páramo, B. Lomonte, J. Pizarro-Cerdá, J.A. Bengoechea, J.P. Gorvel and E. Moreno.

P-143Th: Local tissue damage induced by BAP1, a metalloproteinase isolated from *Bothrops asper* snake venom. A. Rucavado, B. Lomonte, M. Ovadia, J.M. Gutiérrez and J. Núñez.

P-144Th: Pharmacological modulation of edema induced by LYS-49 and ASP-49 myotoxic phospholipases A_2 isolated from the venom of the snake *Bothrops asper* (terciopelo). F. Chaves, G. León, V. H. Alvarado amd J. M. Gutiérrez.

P-145Th: Studies examining the smooth muscle relaxant effects of venom from the inland taipan (*Oxyuranus microlepidotus*) in rat isolated aorta. K.L. Bell, B.K. Kemp, G.A. McPherson and W.C. Hodgson.

P-146Th: Effects of snake venoms on testosterone and some metabolic hormones in male rats. A.K. Tilmisany, K.A.A. Galil and O.H. Osman.

P-147Th: Effects of Russell's viper venom on human erythrocytes *in vitro*. S. Napathorn, M. Tejachokviwat, S. Maneesri, V. Kasantikul and V. Sitprija.

P-148Th: Teratogenic effect of *Naja haje* (Egyptian cobra) venom. M. F. El-Asmar, H. Said, M. Eman, N. Swelam, K. Zaki, H. Nasser, T. Farid, A. Kamal and F. Khalil.

P-149Th: Effect of gamma-toxin from *Tityus serrulatus* scorpion venom on gastric emptying in rats. F. Bucaretchi, C. Chávez-Olórtegui and E.F. Collares.

P-150Th: Scorpion sting epidemic. Montes County, Sucre, Venezuela. L. De Sousa, S. Bónoli, E. Ledezma, P. Parrilla, A. Jorquera and M. Quiroga.

P-151Th: Lung tissue ultrastructural alterations on mice inoculated with *Tityus discrepans* (Buthidae) venom. C. Rodríguez-Rauseo, M. Strauss, L. González, M. Blanco, M. Pulido-Mendez, and A. Rodríguez-Acosta.

P-152Th: Effects of adrenergic blockers and ligation of spleen vessels on hemodynamics of dogs injected with scorpion venom. S. Sofer and A. Tarasiuk.

P-153Th: Tetorodotoxin attracts starfish ("Togemomijigai" *Astropecten polyacanthus*). T. Saito, A. Sakamaki, T. Tamura, K. Kodama and T. Noguchi.

P-154Th: Nitric oxide release by *Phoneutria nigriventer* venom fractions in rat aorta rings. L.C. Oliveira, M.E. De Lima, M.N. Cordeiro, C.R. Diniz and M.L.D. Weinberg.

P-155Th: Endotoxemic-like shock induced by *L. intermedia* spider venom. D.V. Tambourgi, V.L. Petricevich, F.C. Magnoli, S.L. Assaf, S. Jancar and W. Dias da Silva.

P-156Th: Sex-related differences in *L. intermedia* spider venom. K.O. Oliveira, R.M.G. Andrade, A.L. Giusti, W. Dias da Silva and D.V. Tambourgi.

P-157Th: Ontogenic development of *L. intermedia* spider venom. R.M.G. Andrade, K.C. Oliveira, A.L. Giusti, W. Dias da Silva and D.V. Tambourgi.

P-158Th: A comparison of the pharmacological activity of venom from the male and female white-tailed spider (*Lampona cylindrata*). L.D. Rash, E.A. Davis, R.G. King and W.C. Hodgson.

P-159Th: Change of muscle fiber type in fast and slow skeletal muscles induced by ACL myotoxin. H.S. Selistre-de-Araujo, T.F. Salvini, C.C. Morini, E.C.L. Pereira, and C.L. Ownby.

P-160Th: Comparative study of the cytokines profiles induced by *B. asper* and *B. jararaca* envenomation in murine model. V.L. Petricevich, C.F.P. Texeira, D.V. Tambourgi and J.M. Gutiérrez.

P-161Th: Effects of equinatoxins from *Actinia equina* on fish *in vivo* . D. Suput.

P-162Th: Africanized honey bee attack behavior and clinical consequences in humans and dogs: experiences in USA. L.S. Schmidt and J.O. Schmidt.

P-163Th: Anti-cholinesterase activity of 3-alkylpyridinium polymers, isolated from the marine sponge, *Reniera sarai*. K. Sepcic, T. Turk, V. Marcel, A. Klaebe, P. Macek and D. Fournier.

P-164Th: Storage influence on stonefich venom components activity. C. Perrière, C. Le Gall, J.M. Grosclaude, P. Garnier, C. Dewulf and F. Goudey-Perrière.

P-165Th: Stonefish venom neurotoxic effects. C. Perrière, P. Breton, I. Delamanche, C. Le Gall, F. Goudey-Perrière.

P-166Th: The influence of cholesterol in membrane selectivity of antimicrobial peptides from Brazilian frog *Phyllomedusa distincta*. C.V.F. Batista and C.Jr. Bloch

P-167Th: Mexican Institute of Social Security: Epidemiological data on scorpion and snake accidents and their treatment. J.A. Maraboto-Martínez, A. Chávez-Haro, C. García-Willis, M.Rivas and A. Alagón.

II - CONTINUATION SPECIFIC SCIENTIFIC PROGRAM -
(divided into sections A to D)

FRIDAY, 26th SEPTEMBER

RECEPTION AND INFORMATION DESK. 8:30 to 13:00; 14:00 to 17:00

A. - SEVENTH PLENARY LECTURE

08:30-09:30 **Dr. Gerhard Habermehl:** "*Secondary and tertiary metabolites of plant toxins*" (PL-06Fr)

B. - SYMPOSIUM NUMBER 6: "MICROBIAL TOXINS"
09:35-12:25 - Please refer to specific program below

Chairperson: Dr. Leonard Smith
Co-chairperson: Dr. Robert J. Carman

09:35-10:05 - *Clostridium perfringens* toxins, by Robert J. Carman (S-29Fr)
10:05-10:35 - Enterotoxigenic *Escherichia coli*: virulence determinants and vaccines, by Frederick J. Cassels, J.F. Wood, J. van Hamont, B.A. Bell, J. Anderson, M. K. Wolf and C.E. McQueen. (S-30Fr)
10:35-10:55 - Refreshment Break
10:55-11:25 - *Shigella* vaccines: progress and prospects, by Thomas L. Hale, T.S. Coster, C.W. Hoge, C.P. Mallett, A.T. Hartman, L.L. VanDe Verg, D. Cohen and G.H. Lowell. (S-31Fr)
11:25-11:55 - Engineered bacterial superantigen vaccines, by S. Bavari and Robert G. Ulrich. (S-32Fr)
11:55-12:25 - Development of genetically engineered vaccines for botulinum neurotoxins, by Leonard Smith (S-33Fr)

12:25-14:00 - **LUNCH** (on own)

14:00-16:30 - ORAL PRESENTATIONS

C. - TWO SIMULTANEOUS ORAL PRESENTATIONS ON CLONING AND BIOLOGICAL EFFECTS III
(See list in the detailed scientific program below)

ROOM 1: *SUBJECT RELATED TO CLONING AND MISCELLANEOUS*
Chairperson: Dr. Franc Gubensek
Co-chairperson: Dr. Ana Maria Moura-da-Silva

14:00-14:15 Functional mapping of α-dendrotoxin, a blocker of voltage dependent K^+ channels. S. Gasparini, J.M. Danse, A. Lecoq, S. Pinkasfeld, A. Harvey and A. Ménez. (O-71Fr)

14:15-14:30 Processing of pro-tumour necrosis factor-α by venom metalloproteinases and their inhibition. G.D. Laing, A.M. Moura-da-Silva, M.J.I. Paine, J.M.T.J. Dennison, V. Politi, J.M. Crampton and R.D.G. Theakston. (O-72Fr)

14:30-14:45 Expression of jararhagin disintegrin domain in *Escherichia coli*. A.M. Moura-da-Silva, A. Línica, A.S. Kamiguti, P.L. Ho, J.M. Crampton and R.D.G. Theakston. (O-73Fr)

14:45-15:00 Biochemical characterization, cDNA cloning and expression of the fibrinolytic enzyme, lebetase from *Vipera lebetina* venom. E. Siigur, A. Aaspollu, K. Tonismägi, M. Samel, M. Drews, A. Merits, A. Tu and J. Siigur. (O-74Fr)

15:00-15:15 Gene sequence of an Australian tick neurotoxin. S. Masina, M.J. Thurn and K.W. Broady. (O-75Fr)

15:15-15-30 Venoms: a fully interactive database on venomous animals and their venoms. R. Stöcklin. (O-76Fr)

15-30-15:45 Fingerprinting of crude venoms by mass spectrometry: zoological and molecular considerations. R. Stöcklin. (O-77Fr)

15:45-16:00 Acetylcholinesterase from *Elapidae* venom: identification of a new alternative exon and of two residues implicated in the peripheral site. X Cousin, S. Bon, N. Duval, J. Massoulié and C. Bon. (O-78Fr)

16:00-16:15 Inflammatory host response to *Bothrops atrox* venom. T.L. Kipnis, S.F. Barros, V. Petricevich and I. Friedlanskaya. (O-79Fr).

ROOM 2: *SUBJECT MOSTLY TO BIOLOGICAL EFFECTS III*
Chairperson: Dr. David Jones
Co-chairperson: Dr. Yara Cury

14:00-14:15 Central and peripheral mechanisms of the analgesic effect of *Crotalus durissus terrificus* (Cdt) snake venom. R. Gorgi, M. M. Bernardi and Y. Cury. (O-80Fr)

14:15-14:30 Recurrence of coagulopathy following North American pit viper envenomation. G.M. Bogdan, R.C. Dart. (O-81Fr)

14:30-14:45 Characterisation and biological activity of 3-alkylpyridinium compounds from the marine sponge *Reniera sarai*. K. Sepcic, P. Macek, and T. Turk (O-82Fr)

14:45-15:00 Neurotoxic phospholipase A_2-induced epileptic syndrome: a complex model. F. Dorandeu, C. Perrichon, I. Pernot-Marino, P. Lapeyre, S. Aubriot, A. Foquin and G. Lallement. (O-83Fr)

15:00-15:15 Toxic effects of catnip (*Nepeta cataria*) exposure during embriogenetic period in mice. M.M. Bernardi, S. Fernandes, A.L. Zodi, H.S. Spinosa, S.L. Górniak. (O-84Fr)

15:15-15-30 Structure and toxin contents of salivary glands of human body lice. D. Jones and S. Wache. (O-85Fr)

15-30-15:45 Inmunosuppressive effects of wasp venom: blood melanization activity and celullar encapsulation. S.C. Wache and D. Jones. (O-86Fr)

15:45-16:00 Effects of scorpion antivenom on hemodynamic pathophysiology in dogs injected with scorpion venom. S. Sofer and A. Tarasiuk. (O-87Fr)

16:00-16:15 Characterization of a complement-dependent hemolysin from *Bothrops atrox* venom. M.G.M. Freire, E.W. Alves, T.L. Kipnis and W. Dias da Silva. (O-88Fr)

D. - CLOSING CEREMONY (at 16:30)

III.- ABSTRACTS

Plenary (Pl)

Symposium (S)

Oral (O)

12th WORLD CONGRESS IST

PI-01Mo

FUNCTIONAL ARCHITECTURES OF TOXIC PROTEINS : A CLUE FOR DRUG DESIGN?

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Since twenty years, toxic proteins from venomous animals have been extensively studied at the molecular level. This is not surprising since they usually have a small size and are highly stable, making them amenable to both structural analyses and chemical and/or genetic manipulations.

Many toxin architectures have ben elucidated, offering a remarkable structural basis for understanding the diversity of toxic functions produced by venomous creatures. At least three complex situations are observed. First, a group (a family, an order, etc.) of venomous animals produces multiple toxins with the same fold, suggesting functional divergence on a conserved structural theme. Second, animals from distinct phyla produce toxins with similar functions but different folds, suggesting a functional convergence of the folds. Third, animals from distinct phyla produce toxins with similar functions and folds, suggesting both structural and functional convergences. In addition, toxin folds are present in various tissues other than venom glands and even in plants, where they usually exert quite distinct functions. Toxin templates might follow various though related evolutionary strategies to generate and accommodate multiple functions.

The advent of protein engineering makes it now possible to elucidate the topographies by which toxin folds exert their functions. Already, some general features associated with these topographies are emerging. Thus, these sites (i) are homogeneous surfaces that are spread on variable regions of a scaffold; (ii) include less than 10 residues, with 3 or 4 most critical side chains; (iii) possess a core of conserved residues surrounded by variable residues which provide toxins with unique specificities and high affinities for their receptors.

Understanding of the functional anatomy of animal toxins has at least two practical consequences. First, toxin scaffolds are attractive templates to engineer new functions. This aspect will be illustrated by some examples. Second, an identified functional topography may help to design new ligands. It will be shown that a novel compound has been engineered to diagnose allergy to myorelaxants, using the functional anatomy of a snake toxin as a « lead compound ».

In conclusion, elucidation of functional architectures of animal toxins has opened new vistas to better understand their molecular evolution, to engineer new functions and to design novel drugs.

PI-02Mo

FOUNDING HISTORY OF THE INTERNATIONAL SOCIETY ON TOXINOLOGY (IST)

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A wise philosopher once said, "History is a beacon into our future". The history of our Society had its beginnings in an idea. An idea, expressed resourcefully by Mrs. Eleanor Buckley of Wyeth Laboratories to set up an international meeting for scientists who were interested in the venoms and poisons produced by animals. This gave to the *First International Conference on Venoms*, held in San Francisco in 1945. It was not until 1960, however, that I wrote to approximately 20 scientists throughout the world concerning a society and journal. Of the 18 that replied, 17 expressed an interest in such a venture, and in 1962 the I.S.T. and journal *Toxicon* were formed. The presentation reviews the trials and tribulations of the founding of the Society and its many humorous adventures.

PI-03Tu

SCORPION TOXINS: TOOLS FOR STUDYING K^+ CHANNELS

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Potassium channels comprise a large family of proteins that share in common the property of being highly selective for mediating K^+ movement. Molecular biology has been pivotal in the identification of many K^+ channel subfamilies due to the high conservation of a signature sequence that is believed to contribute to the formation of the potassium-selective pore. The physiologic role that many of these proteins play in cell function is, however, still largely uncharacterized. To aide in such studies, high-affinity and selective blockers of a given K^+ channel must be developed. Scorpion venoms contain a variety of peptidyl blockers directed against K^+ channels. These peptides have been purified to homogeneity, their primary amino acid sequence determined, and, for some of these agents, the three-dimensional structure has been obtained in solution by NMR techniques. The most studied of these blockers is charybdotoxin (ChTX), a 37 amino acid peptide containing three disulfide bridges, isolated form *Leiurus quinquestriatus* var. *hebraeus* venom. ChTX binds to the outer vestibule of certain K^+ channels and blocks ion conduction by physical occlusion of the pore. ChTX has been useful as a tool in the purification of the smooth muscle high-conductance Ca^{2+}-activated K^+ channel, in determination of this channel's subunit composition, and in the development of the channel's molecular pharmacology. With the use of two other related peptides, margatoxin and noxiustoxin, it has been possible to characterize the role that the voltage-gated K^+ channel, $K_v1.3$, plays in human T cell signal transduction processes. In addition, site-directed mutagenesis studies of peptides and the channels which they inhibit have provided insights into the architecture and dimensions of the vestibule of K^+ channels.

PI-04Tu

A FAMILY OF RECEPTOR FOR VENOM PHOSPHOLIPASES A_2

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Venom phospholipases A_2 (vPLA_2's) display a large spectrum of toxic effects including neurotoxicity, myotoxicity, hypotensive, anticoagulant and proinflammatory effects. We have shown that these different types of effects are apparently linked to the existence of a variety of a very high affinity receptors (K_d values as low as 1.5 pM) for these toxic enzymes. On the other hand, mammalian secretory PLA_2's (msPLA_2's) are now implicated in many biological functions besides digestion, such as airway and vascular smooth muscle contraction, cell proliferation, and a variety of diseases associated with inflammation such as rheumatoid arthritis, endotoxic shock, respiratory distress syndromes and with cancer. Several different types of receptors (N and M) have been identified for vPLA_2's and one of them (180 kD called M) has been cloned in rabbit and man and sequenced. It is a membrane protein with a N-terminal cystein rich domain, a fibronectin type II domain, eight repeats of a carbohydrate recognition domain, a unique transmembrane domain and an intracellular C-terminal domain. When expressed in transfected cells it binds msPLA_2's (synovial PLA_2, K_d~1-10 nM) indicating that the receptors for toxic PLA_2 are the normal targets of endogeneous msPLA_2 involved in a variety of diseases. Residues within or close to the Ca^{2+}-binding loop of sPLA_2 are crucially involved in the binding step although the presence of Ca^{2+} which is essential for the enzymatic activity is not required for binding to the receptor. The molecular domain in charge of vPLA_2 binding in the M-type receptor has been identified. The M-type receptor is an endocytic receptor. N-type receptors were first identified in brain membranes. They have a particularly high affinity for neurotoxic PLA_2's and are made of protein subunits of 36-51 and 85 kDa. Bee venom PLA_2 has a particular high affinity for this type of receptor and amino-acids residues of this PLA_2 which are involved in binding to N-type receptors have been identified.

PI-05Th

BLACK WIDOW SPIDER TOXINS: THE PRESENT AND THE FUTURE

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There are very few natural neurotoxins that can stimulate the transmission secretion from nerve terminals. Among them most known first of all are α-latrotoxin (α-LTX) and some other high molecular mass toxins from the venom of the black widow spider *Latrodectus mactans tredecimguttatus*. α-LTX provokes a massive transmitter release from different nerve endings of vertebrates, whereas other toxins increase the secretion process either in the insect or in crustacean. The cDNAs encoding the putative α-LTX and two latroinsectotoxins (α-LIT and δ-LIT) precursors were cloned and sequenced. These toxins are polypeptides of about 1000 amino acids and share a high level of amino acid identity. Analysis of amino acid sequences of the three toxins reveals the central regions being almost entirely composed of a series ankyrin-like repeats. The δ-LIT cDNA was expressed in bacteria and the resulting protein was highly toxic for insects. Taking into account the size and multifunctional properties of latrotoxin its molecule can be divided into several functional domains. Immunochemical experiments indicated the presence of distinguishable functional domains in the α-LTX molecule responsible for ionophoric and secretogenic actions. Structural and functional information available reveals the common structural latrotoxin organization, although it cannot currently clear explain different phylum specific toxicities. Several attempts were made to characterize and isolate α-LTX receptor components. The existence of Ca^{2+}-dependent and Ca^{2+}-independent binding proteins was found in the presynaptic membrane preparations.

PI-06Fr

SECONDARY AND TERTIARY METABOLITES AS PLANT TOXINS

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Besides the so-called primary metabolites necessary for the common metabolism, plants have developed during their evolution a broad variety of secondary metabolites not essential for their daily life. Nearly all of these compounds are bioactive, and therefore, depending on their concentration, toxic for humans as well as for animals. Chemically, these toxins belong to different classes: Alkaloids usually act as neurotoxins, steroid-glycosides as cardiotoxins, and terpenes as substances affecting the complete intestinal system including liver and kidney. There have been many theories on the purpose of those substances, the most plausible one being a protection against consumption because of their burning or bitter taste. Nevertheless animals have not learned to avoid such plants, and we observe many cases of poisoning. The interaction with fungi imperfecti or other microorganisms is not clear. While some of these substances are active against microorganisms, other microorganisms have learned to live together with plants in a kind of symbiosis. One striking example is the symbiosis between the shrub *Baccharis coridifolia* and the fungus *Myrothecium verrucaria*, in which the plant receives its toxin from the fungus. We propose the name "tertiary metabolites" for such toxins derived from external sources. There are also plant-plant interactions in which such substances e.g. terpenes are used for promoting or inhibiting plant growth.

12th WORLD CONGRESS IST

S-01Mo

PHOSPHOLIPASE A_2 TOXINS: DIVERSITY IN STRUCTURE AND FUNCTION

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Phospholipases A_2 are enzymes which hydrolyze the 2-acyl group of phospholipids and release fatty acids and lysophospholipids. Based on primary sequence data, the secreted PLA_2s can be categorized into at least three types. Type I includes enzymes from mammalian pancreas and venoms from snakes in the families Elapidae (cobras and kraits) and Hydrophidae (sea snakes) and from human spermatozoa. Type II includes enzymes in the venoms of snakes in the family Viperidae, subfamily Crotalinae (pit vipers) and Viperinae (true vipers) as well as some mammalian cell types, including platelets, gastric mucosae and vascular endothelium. Type III are enzymes from the European honeybee, the Gila monster and the Mexican beaded lizard. Although very similar in structure, these enzymes show remarkable diversity in their functional properties. Neurotoxicity, myotoxicity, anticoagulant and proinflammatory are some of the biological effect of these toxic enzymes. We have examined some Type I, II and III PLA_2 toxins to determine if there is a correlation between structure (primary, secondary and tertiary) and myotoxic function. Neither porcine pancreatic PLA_2 (5.0 μg/g) nor β-bungarotoxin (0.01 and 0.07 μg/g), both Type I PLA_2s were myotoxic in mice. However, several Type I PLA_2s from Elapid venoms did cause myonecrosis. These included PLA_2s from *Naja naja atra*, *Naja naja kaouthia*, and *Naja nigricollis* venoms as well as scutoxin from *Notechis scutatus scutatus* venom. Two Type II myotoxis, i.e., ACL myotoxin from *Agkistrodon contortrix laticinctus* venom and CVV myotoxin from *Crotalus viridis viridis* venom, induced myonecrosis. Also one Type III PLA_2, from honeybee (*Apis mellifera*) venom, induced myonecrosis in the same type of experiment.

S-02Mo

SKELETAL MUSCLE DAMAGE CAUSED BY MYOTOXIC PHOSPHOLIPASES

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Although first identified as a major class of toxins by Harris et al. in 1975, it is only recently that the mode of action of the phospholipase myotoxins has begun to become clear. The results of several groups suggest: 1) the toxins bind to an as yet unidentified 'acceptor' in the plasma membrane of the muscle fibre; 2) the association of the hydrolytic site of the toxin and the lipid substrate leads to the hydrolysis of the membrane lipids; 3) an increase in membrane fluidity, the lost of ion gradients (Johnson & Ownby, 1994), and the appearance of small lesions in the plasma membrane (Dixon & Harris, 1996) leads to areas of local hypercontraction. These place the intervening, weakened plasma membrane under great strain and the intervening membrane is torn apart (Dixon & Harris, 1996).

This process is initiated in minutes and is probably completed by1-3 hrs. It is followed by the hydrolysis of key components of the cytoskeleton and sarcomeres (especially desmin and titin) over the following period of 3-6 hours (Vater et al. 1992). The hydrolysis of the cytoskeleton results in the disruption of the sarcomeres and allows the enzymatic degradation of the major contractile proteins actin and myosin (Faiz et al. 1995).

This sequence of events fits available data and could easily reach the status of received wisdom, but it cannot be left unchallenged. Significant questions remain unanswered: What are the 'acceptors' in the plasma membrane? Which part of the toxic molecule is responsible for myotoxicity? How do the toxins gain access to the C2 side chains of the membrane phospholipids, buried as they are in the hydrophobic region of a lipid bilayer? How can the Asp-49-Lys variants of the toxic molecules be myotoxic when they are devoid of hydrolytic activity? Which enzymes are activated to lead to the breakdown of the major structural and functional proteins of the skeletal muscle fibres?

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S-03Mo

Lys49 PHOSPHOLIPASE A_2 MYOTOXINS LYSE CELL CULTURES BY TWO DISTINCT MECHANISMS

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Three Lys49 phospholipase A_2 (PLA_2) myotoxins from *Agkistrodon contortrix laticinctus* (ACLMT), *Bothrops jararacussu* (bothropstoxin-I) and *Bothrops asper* (myotoxin II) snake venoms were enzymatically inactive on artificial substrates, yet hydrolyzed phospholipids in cell cultures. Bothropstoxin-I treated with *p*-bromophenacyl bromide was no longer active on cell cultures, suggesting the toxin, not tissue PLA_2, hydrolyzed the phospholipids. The role of this enzymatic activity in necrosis was examined in detail for ACLMT. The NB41A3 cell line was found to be highly sensitive to lysis by ACLMT by two separate mechanisms. The first mechanism, predominant at lower concentrations of ACLMT (0.1-0.5 μM) and long incubation periods (24 hr), was antagonized by methylprednisolone (MePDN) (50 μM). The second was predominant at higher concentrations of toxin (1-5 μM) incubated over a short period (1 hr) and was not antagonized by MePDN. There was no correlation between enzymatic activity and toxicity at the higher concentrations (5 μM; 1 hr) when the enzymatic activity of ACLMT was compared with a noncytolytic PLA_2 from *Naja naja atra* venom (1 μM). However, over a 24 hr period, triglyceride formation relative to fatty acid formation was about 40-fold greater for ACLMT, suggesting the two enzymes act on substrates associated with different cellular compartments under this condition. MePDN did not alter fatty acid production or substrate specificity of ACLMT, but did alter the subsequent accumulation of fatty acids into triglycerides. This is the first clear demonstration of two mechanisms of myonecrosis dependent on toxin concentration and that MePDN-sensitive triglyceride accumulation induced by low concentrations of a PLA_2 correlates with myotoxicity.

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S-04Mo

ANTICOAGULANT PHOSPHOLIPASE A_2: FROM SNAKE VENOMS TO HUMAN ENZYMES

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Extracellular (*i.e.* secreted) forms of phospholipase A_2 ($sPLA_2$) are abundant in the secretions of exocrine glands, such as pancreas and venom glands from snakes, lizards, bees, etc. In mammals, $sPLA_2$ is released from several types of cells, such as blood platelets or macrophages, upon their activation. These enzymes are homologous polypeptides of 13 to 14 kDa which, in addition to their role in digestion, can cause a wide variety of pharmacological effects, including anticoagulant action.

Several investigations performed to study the mechanism of action of anticoagulant $sPLA_2$ from snake venoms, showed that these enzymes inhibit blood coagulation with different potencies. Furthermore, it appeared that some of them prevent clot formation by hydrolysing phospholipids, since their catalytic activity is essential for the expression of their anticoagulant activity. Other venom $sPLAs_2$ inhibit prothombinase complex by a mechanism independent of their enzymatic activity, which might be due to their binding to either factor Va or factor Xa. On the other hand, it has been suggested that residues 54 to 77 of snake venom anticoagulant $sPLAs_2$ correspond to the "anticoagulant region".

Recent investigations demonstrated a significant anticoagulant action of human group II $sPLA_2$ secreted by blood platelets. This effect, which does not require the enzymatic activity of the enzyme, is due to the inhibition of factor Xa, possibly at its interaction site with factor Va. Furthermore, a peptide corresponding to residues 51 to 74 of human platelet $sPLA_2$ mimics the action of the whole PLA_2 on factor Xa.

S-05Mo

AT THE INTERFACE - CRYSTAL STRUCTURES OF PHOSPHOLIPASE A_2s

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Lys49-Phospholipase A_2 homologues from the venoms of *Bothrops* species are considered to be catalytically inactive due to an Asp49→Lys substitution. However, they retain the ability to disrupt the integrity of lipid membranes by a Ca^{2+}-independent mechanism. The crystal structures of several dimeric PLA_2 homologues have been determined in our laboratory, in which the dimer interfaces are defined by contacts between the N-terminal α-helical regions and the tips of the β-wings of partner monomers, thus permitting ready access to the lipid binding and "catalytic" sites. Significant differences in the relative orientations of the monomers have been observed resulting in "open" and "closed" dimer conformations. Spectroscopic investigations in solution correlate these conformational differences with changes in the intrinsic fluorescence emission of the single tryptophan residues located at the dimer interface. These transitions may be of relevance in the Ca^{2+}-independent membrane damaging activity. We have extended our structural investigations using these proteins as model systems to study the binding of natural lipid substrates, fatty acids and detergents which are providing further insights into the molecular basis of the function of these enzymes. This structural information will be potentially important in the design of new PLA_2 inhibitors. Lys49-PLA_2 homologues posses myotoxic and anti-coagulant activities and additional studies have focussed on correlating amino acid sequence and structural information with pharmacological activities.

Acknowledgements to FAPESP, CNPq for financial assistence.

S-06Mo

Effect of Site Directed Mutagenesis on the Activity of Recombinant Trimucrotoxin, a Neurotoxic Phospholipase from *Trimeresurus mucrosquamatus* Venom.

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Trimucrotoxin, the basic phospholipase A_2 from venom of Taiwan habu *Trimeresurus mucrosquamatus* is neurotoxic and myotoxic. Its amino acid sequence is 80% identical to that of crotoxin B (the basic subunit of crotoxin) and it is 1/3 as toxic as crotoxin for chick biventer cervicis neuromuscular tissue. To investigate the amino acid residues responsible for the neurotoxicity of trimucrotoxin, we site-directed mutated some interface-recognition residues of the enzyme. Herein we report the effects of mutations at Asn6. The wild-type and the mutants were expressed in *E. coli* as fusion-proteins and activated after refolding by factor Xa cleavage. Enzymatic activities and neurotoxicity toward the chick tissue of four trimucrotoxin mutants (N6A, N6E, N6R and 6E7T8L) were compared with those of the wild type. Mutants N6A and N6E retained more than half of the original enzymatic activity but their neurotoxicities reduced to 33% and 25%, respectively, that of the recombinant wild type (which is as active as the toxin isolated from the venom). Mutants N6R and 6E7T8L retained 20-25% of the enzyme activity toward the anionic micellar substrate but inactive toward the zwitterionic micellar substrate, and their neurotoxicities were less than 8% of that of the wild type. Thus, it is likely that the highly conserved residues 6-8 in these crotalinae neurotoxins are important for their binding with neuronal binding sites as well as the enzyme specificity toward aggregated substrates.

S-07Mo

THE Bov-B LINE FOUND IN *VIPERA AMMODYTES* TOXIC PLA_2s IS WIDELY SPREAD IN SNAKE GENOMES.

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Ammodytoxin C, a presynaptically toxic PLA_2, and ammodytin L, a myotoxic PLA_2 homologue, are group II PLA_2s, specifically expressed in the venom gland. The typical genes of type II PLA_2s consist of 5 exons separated by 4 introns. In the 4th intron of toxic PLA_2s genes we found an ART-2 retroposon sequence (1), the origin of which was unknown. Recently it has been shown to be a 3' part of a LINE (long interspersed nuclear element) and not a SINE (short interspersed nuclear element) as believed earlier. Consequently ART-2 retroposons are now designated as Bov-B LINEs. These transposable DNA elements are present in a very high number of copies and, once inserted in genome, remain stable, so that they may serve as evolutionary markers (2). The Bov-B LINEs, believed to be specific for ruminants, were found in viperid genomes and in all other snake genomes tested, except in sea snake *Laticauda semifasciata*. The degree of sequence divergence among Squamata and Ruminantia indicates their amplification during the Mesozoic era in Squamata about 100Myr ago, and horizontal transfer to the ancestor of Ruminantia during the Eocene, about 50 Myr ago. Bov-B LINEs represent a fossilized record of a major genetic event preceeding the radiation of Squamata and true ruminants. These data provide compelling evidence that Bov-B LINEs mediated an extensive radiation of both orders. The original designation »Bov-B« seems thus inadequate and may need revision when its true distribution will be established.

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S-08Tu

WHAT CAN TOXINS TELL US FOR DRUG DISCOVERY?

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Toxins are of interest in drug design because the toxins provide 3-dimensional templates for creating small molecular mimics with interesting pharmacological properties. Toxins are also useful in drug discovery because they can be used as pharmacological tools to uncover potential therapeutic targets. With their high potency and selectivity, toxins are often more useful in functional experiments than standard pharmacological agents. We have used two groups of neurotoxins, the dendrotoxins and the muscarinic toxins (MTs), to explore the involvement of subtypes of potassium ion channels and muscarinic receptors, respectively, in processes involved in cognition and the changes in neuronal properties with aging.

From our current work, quantitative autoradiographic studies with radiolabelled dendrotoxins reveal widespread distribution of binding sites throughout rat brain sections, but few differences between adult and aged rats. However, displacement studies with toxin K, which preferentially binds to the Kv1.1 subtype of potassium channel, show the selective loss of such sites in regions of the hippocampus and septohippocampal pathway with aging. MTs have been tested for effects on performance of rats in memory paradigms. MT2, which activates M1 receptors, improves performance of rats in a step-down inhibitory avoidance test, whereas MT3, which blocks M4 receptors, decreases performance when given into the hippocampus. This is the first clear demonstration of a role for M4 muscarinic receptors in cognition.

S-09Tu

FROM NOXIUSTOXIN TO *SHIVA-3*, A PEPTIDE TOXIC TO THE SPOROGONIC DEVELOPMENT OF *Plasmodium berghei*

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Noxiustoxin (NTX), the first described toxic peptide specific for K^+-channels has been throughly studied, including the determination of its 3-D structure (*Biochem.* 34:16563-16573, 1995). Synthesis of peptides corresponding to fragments of its primary structure and the gene coding for the native and site-directed mutagenized recombinants were prepared and assayed (*Biochem. & Mol. Biol. Int.* 37:527-535, 1995; *Toxicon* 34: 1413-1419, 1996). NTX has been a model peptide for the discovery and study of at least 20 different peptides with similar activities (*Biochem.* 36:2649-2658, 1997). However, still more important, it served as a model molecule for the design and assay of Shiva-3, a synthetic peptide similar to cecropins, shown to inhibit the development of the sporogonic phase of *Plasmodium berghei* (*Exp. Parasitol.*80:596-604,1995). Shiva-3, a 38 amino acid long peptide, had its 3-D structure determined by ^{1}H-NMR. A synthetic Shiva-3 gene was constructed by recursive PCR methodology and expressed using the vector pGEX2T as a hybrid protein between the Glutathion-S-transferase at the N-terminal and Shiva in the C-terminal. The recombinant was shown to be effective killing bacteria and *Plasmodium berghei* (responsible for a murine version of malaria). The present work is aimed at producing a transgenic mosquito that carries the message for synthesis and excretion of Shiva-3 and similar peptides, in the midgut of mosquitoes, in an attempt to control the spreading of human malaria.

Acknowledgments: UNDP/World Bank/WHO special TDR grant (ID-930812), and Howard Hughes Medical Institute (75197-527107).

S-10Tu

Inhibition of Mouse Acetylcholinesterase by Fasciculin: Crystal Structure of the Complex and Mutagenesis of Fasciculin

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Fasciculins are members of the superfamily of three-fingered peptidic toxins from *Elapidae* venoms. They selectively inhibit mammalian and electric fish acetylcholinesterases (AChE) with Ki values in the pico- to nanomolar range. Kinetic studies performed in solution indicate that fasciculin does not totally occlude ligand access to the active site of AChE, but rather binds to a peripheral site of the enzyme to inhibit catalysis, perhaps allosterically.

The crystal structure of the Fas2-mouse AChE complex delineated a large contact area consistent with the low dissociation constant of the complex; the Fas2 and AChE residues participating in the binding interface were unambiguously established, and major hydrophobic interactions were identified [1]. The structure however suggests that fasciculin totally occludes substrate entry into the catalytic site of AChE, and does not reveal to what extend each contact between Fas2 and AChE contributes to the overall binding energy. New probes, aimed at analyzing the complex conformation and the individual contributions of the fasciculin residues to the complex formation, were generated by site-directed mutagenesis of a synthetic Fas2 gene [2]. A fully processed recombinant fasciculin, rFas2, that is undistinguishable from the natural, venom-derived Fas2, was expressed in a mammalian system; fourteen mutants, encompassing 16 amino acid residues distributed among the three loops (fingers) of Fas2, were designed based on both the kinetic and structural data, and analyzed for inhibition of mouse AChE.

The determinants identified by the structural and the functional approaches do coincide, but only a few of the many residues which make up the overall interactive site of the Fas2 molecule provide the strong interactions required for high affinity binding and enzyme inhibition.

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S-11Tu

STRUCTURE-ACTIVITY RELATIONSHIP OF INSECTICIDAL SCORPION NEUROTOXINS AND THEIR USE FOR THE DESIGN OF NOVEL INSECTICIDES

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Our study is motivated by the need to develop new means and approaches to the design of future, environmentally-safe, insecticides. We aim at clarifying the molecular basis of the anti-insect selectivity of scorpion neurotoxins and increasing the killing efficacy of entomopathogenic baculoviruses by their engineering with the corresponding scorpion genes. A large collection of cDNA clones and an efficient bacterial expression system enabling mutagenesis have been established. By using a multidisciplinary approach combining point mutagenesis, binding and electrophysiological assays, circular dichroism spectroscopy and NMR, we elucidated the toxic surface of the highly insecticidal toxin LqhalT from the Israeli yellow scorpion. Mutants with elevated toxicity or with improved anti-insect/anti-mammalian toxicity ratio have been produced. In parallel, we constructed recombinant AcNPV baculoviruses expressing three pharmacologically distinct scorpion toxins in insect cell lines. The insecticidal efficacy (lethality and particularly stopping of feeding) of the engineered viruses improved considerably the effects obtained with wild-type viruses upon infection of Heliothis armigera and Spodoptera littoralis larvae.

S-12Tu

AN N-TYPE NEURONAL CALCIUM CHANNEL BLOCKER IN THE VENOM OF *CONUS MAGUS* DEFINES A NEW CLASS OF PHARMACOTHERAPEUTIC AGENT

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The venoms of piscivorous marine snails of the genus, *Conus*, are rich sources of bioactive compounds that interact with molecular targets in both fish and mammalian tissues. Peptides that block voltage-sensitive calcium channels (VSCCs) have been isolated from several *Conus* species. These compounds, termed ω-conotoxins, range in size from 24 to 29 amino acids, are polycationic, and contain a characterisitc arrangement of six cysteine residues linked by three disulfide bridges. One of these, ω-conotoxin MVIIA, selectively blocks mammalian N-type neuronal VSCCs. N-type VSCCs regulate depolarization-induced calcium entry into neurons, and by this action control a variety of intracellular calcium-dependent processes including the modulation of neuronal excitability, release of neurotransmitters, activation of second messenger systems, and gene transcription. Non-clinical studies in our laboratory have shown that the synthetic form of ω-conotoxin MVIIA, SNX-111, possesses both analgesic and neuroprotective properties. When administered intraspinally, SNX-111 produces significant, dose-dependent antinociceptive effects in animal models of acute, persistent, and neuropathic pain. These effects are mediated, in part, by interruption of the transsynaptic propagation of nociceptive signals from primary spinal afferents to the ascending sensory pathways. Other studies have shown that intravenously-administered SNX-111 is consistently neuroprotective in animal models of both global and focal cerebral ischemia, indicating that components of calcium influx and neurotransmitter release regulated by N-type VSCCs are importantly implicated in ischemia-induced neuronal degeneration. Taken together, these findings suggest that selective N-type neuronal VSCC blockers may be useful in the treatment and management of pain and ischemic brain injury. Accordingly, safety and efficacy studies of SNX-111 have been initiated in humans. Clinical findings to date have been consistent with the non-clinical data.

S-13We

FISH POISONING DUE TO TOXINS OF MICROALGAL ORIGINS IN THE PACIFIC

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Ciguatera is the fish poisoning most prevalent in the tropical Pacific. The planar structure of the major toxin named ciguatoxin was determined in 1989, but the extremely small amount and the poor crystallinity of the toxin hampered the elucidation of the absolute stereochemistry. Recently, we succeeded to determine the absolute configuration of the toxin. Many ciguatoxin congeners were also isolated and the structures were determined. To facilitate screening of toxic fish, receptor replacement assays by a chemoluminescent derivative is proposed. The absolute configuration of maitotoxin, which is characteristic to herbivorous fish, was also determined. The epiphytic dinoflagellate *Gambierdiscus toxicus* was unambiguously determined as the origin of these toxins.
Clupeotoxism is a highly fatal poisoning, though rare in occurrence. Palytoxins were identified as the cause, and a dinoflagellate *Ostreopsis siamensis* was confirmed as the toxin source. Toxins were distributed also in other marine animals.
Shark poisoning has been distinguished from ciguatera by the severity of the symptoms and high mortality rate. Two new toxins were isolated and named carchatoxins. Toxin chemistry is under way.

S-14We

CYTOTOXIC AND ICHTHYOTOXIC COMPOUNDS FROM MARINE OPISTHOBRANCHIA AND SOFT CORALS

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Marine invertebrates are considered to possess allelochemicals for protection against the attack of predators and microorganisms. Especially, Opisthobranchia and soft corals, which have no physical defense system, are expected to possess strong biologically active compounds used for chemical defense. We have been searching these compounds from marine invertebrates with the aim to find new lead compounds for medicines. In this paper, we report the structure and biological activities of compounds obtained from Opisthobranchia (1) and soft corals (2). From Aplysiacea (*Aplysia kurodai* and *A.parvula*) and from Nudibranchia (*Chromodoris inornata* and *C.obsoleta*), belonging to the Opisthobranchia, new isoprenoids, halogenated monoterpenoids, halogenated acetogenins, sesterpenoids and spongian diterpenoids were isolated and characterized. On the other hand, from the soft corals (*Litophyton* sp., *Xenia elongata*, *Cladiella sphaeroides*, *Lobophytum schoedei* and *Sinularia nanolobata*) belonging to the Alcyonaria, new eunicellin-type, xenicane-type, cembrane-type and amphilectane-type diterpenoids were obtained.Terpenoids from *A.kurodai*, *C.inornata*, *C.obsoleta* and *S.nanolobata* showed cytotoxic activity towards murine lymphoma L1210 cells and to human epidermoid carcinoma KB cells *in vitro*. On the other hand, an acetogenin from *A.parvula* and terpenoids from *Litophyton* sp., *X.elongata*, *L.schoedei* exhibited ichthyotoxicity to mosquito fish. Furthermore, terpenoid from *C.sphaeroides* and *L.schoedei* displayed toxicity to brine shrimp.
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S-15We

CIGUATOXINS ON SERRANIDAE AND LUTJANIDAE FISH OF BAJA CALIFORNIA SUR, MEXICO.

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ABSTRACT During spring season over the years 1993-1996, human poisoning events were manifested at El Pardito island and San Evaristo point in La Paz Bay area in Baja California Sur, México. Gastrointestinal, neurological and cardiovascular disorders follow the consumption of liver from *Serranidae* and *Lutjanidae* carnivorous fish. In spring 1996, adult specimens, one of *Mycteroperca prionura* and one of *Lutjanus colorado* were obtained from the same area. Liposoluble toxins were extracted from the liver (Gamboa *et al*, 1992) and evaluated by mouse bioassay (Lewis *et al*, 1991). Mice developed clinical signs compatible with ciguatera. The sample from *M. prionura* reached 3.42 µg of ciguatoxin equivalents per kg of tissue. The extracts were analyzed in HPLC (Legrand *et al.* 1992) and samples from toxic fish showed chromatography peaks absent in negative controls. Furthermore, their chromatography behavior corresponds to fractions described as Ciguatoxin-1. In spite of that the precursor organism of such toxins in the area has not been identified yet, it is noteworthy that blooms of the dinoflagellate *Prorocentrum mexicanum* and the cyanobacteria *Oscillatoria erythraea* were reported in the area.
Ref: Gamboa *et al*, 1992 and Legrand *et al.* 1992, in Toteson T.R. (ed) "Proceedings of the Third International Conference on Ciguatera" Quebec: Polysciences Publications. Lewis *et al*, 1991 Toxicon 29:1115-1127.
Key Words: Ciguatoxins, *Serranidae*, *Lutjanidae*, *Mycteroperca prionura*, *Lutjanus colorado*, carnivorous fish, ciguatera, HPLC, mouse bioassay

S-16We

OCCURRENCE, IMPACT AND MANAGEMENT OF CYANOBACTERIAL BLOOMS AND TOXINS IN EUROPEAN FRESHWATER AND MARINE ENVIRONMENTS

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In agreement with toxicity assessments elsewhere in the world, the cyanobacterial blooms, scums and mats in European fresh-, brackish and marine waters have a high probability of producing toxins. Cyanobacterial toxins present acute and chronic hazards to human and animal health. Acute poisonings attributed to cyanobacterial toxins in Europe include wild, domestic and farm animal deaths, fish- and bird-kills. Examples of human health problems attributed to cyanobacterial toxins have emerged from studies in Australia, Africa, N. America and China, with the deaths of over 50 haemodialysis patients after exposure to cyanobacterial hepatotoxins (microcystins) in Caruaru, Brazil 1996 being the most recent known human incident. In the U.K., human health problems have occurred after accidental ingestion of microcystin-containing cyanobacterial scum and skin contact during water-contact sports.
Whilst about 60 cyanobacterial toxins have been identified, including hepatotoxins, neurotoxins and cytotoxins, bioassay-guided analyses and toxicity assessments indicate the presence of additional bioactive products of cyanobacteria, including inflammatory agents and compounds which are toxic to aquatic biota. The high probability that individual blooms, mats and scums contain levels of known toxins, which would present risks to mammalian health, justifies the need for a "precautionary principle" which now operates in several European countries, i.e. such cyanobacterial growths are assumed to be toxic, unless analysed and found to be otherwise. Requirements exist for increased knowledge of the occurrence of cyanobacterial toxins, toxicity assessment, toxin analysis and toxin removal, unless waterbodies with a high demand e.g. for drinking and recreation are to be restricted for use. Guideline values for these toxins in drinking waters are being derived and policies for recreational waters affected by cyanobacterial blooms are being developed in several European countries. Multiple approaches to anticipate and reduce the adverse effects of these toxins on water resources and supplies are briefly reviewed.

S-17We

OCCURRENCE AND SEQUESTRATION OF TOXINS IN FOOD CHAINS

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Animals may acquire toxicity by absorbing toxic compounds from their food, e.g. plants or other animals. Sequestration and accumulation of toxins may provide protection from predators, which learn to avoid this prey because of unpleasant experiences such as striking or bitter taste. This is a common phenomenon in marine as well as in terrestrial ecosystems. Moreover, toxins may enter food chains where they accumulate reaching high, often lethal concentrations. Adaptation and resistance of the animal to the toxin´s action are inescapable conditions.

Toxin accumulation is of particular importance, when human food production is concerned. Ciguatoxin deriving from a marine benthic dinoflagellate accumulates at first in herbivorous, then in carnivorous fish causing ciguatera in man. Palytoxin which had been primarily detected in marine zoanthids (Palythoa sp.), also occurs in a wide range of other animals, e.g. in crustaceans, worms etc., but also in fish, which feed on crustaceans and zoanthids as well. Toxins from blue-green algae (cyanobacteria) like microcystin occur regularly in freshwater lakes and ponds, predominantly associated with algal blooms. It is very likely that these toxic compounds are also accumulating in higher organisms. However, limits of their detection in biological samples other than algae prevented similar studies. Insects are highly adaptive to plant toxins, which are sequestered during feeding and eventually stored in their body. They provide interesting models to study the mechanism which lead to toxin-resistance enabling the dissemination of these compounds in the food web.

S-18Th

THE DEVELOPMENT AND THE USE OF THE IMMUNOTHERAPY IN AFRICA

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The immunotherapy was recently developed due to the improvement of purification techniques of the antivenom and results of the research in toxicology and pharmacology. The utilization of highly purified IgG fragments leads to a best tolerance and a higher efficacy. The comprehension of the molecular mechanism aiming the elimination of the venom leads to an improvement of the therapeutic protocol.

Snake envenomations constitute in Africa, as in a many tropical countries, an important public health problem. A recent compilation of the literature, the main source of health information in this part of the world, showed that the annual incidence of snakebites was about 1 million and the mortality by 50,000 deaths per year. In besides, the health infrastructure is deficient and snakebites are not correctly managed. Less than 10% of the antivenom needs are effectively covered and, probably in most of envenomations, used at insufficient doses.

The development of immunotherapy in Africa is necessary to reduce the mortality. The injection of purified IgG through intravenous route is well tolerated and efficient. This has already allowed to dramatically decrease the mortality. The identification of simple clinical and/or biological indicators permitted to improve both the supervision and the management of envenomations. The utilization of adjuvant therapy should allow to reduce doses of antivenom and to increase the efficiency of the antivenom while reducing its cost. The development of new financial procedures for the management of snakebites are necessary to generalize their use. Finally, the training of the health workers for these new techniques is of great importance.

S-19Th

ENVENOMING AND ANTIVENOM USE IN AUSTRALIA

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Australia has a diverse and rich venomous fauna, both terrestrial and marine, including some of the most venomous species in each class. Antivenom is the principal therapy for the majority of medically significant envenomings and is currently supplied through a single source, CSL Ltd, Melbourne. Cases of envenoming reported to Australian poisons information centres (PICs) are dominated by spiderbite and insect stings, respectively accounting for 53.7% and 39.3% of all bite/sting calls. Marine animal bites/stings/poisonings account for only 4% of PIC calls in this category, while snakebites account for a mere 3% (still at least 400 calls/yr). Because most PIC calls are from the public, not doctors/hospitals, snakebite in particular is under-represented.

The author has recently reviewed antivenom usage in Australia. Snakebite affects between 1,000 to 3,000 people per year, with an average annual mortality of about 2 cases. Brown snakes (genus *Pseudonaja*) cause the majority of deaths (and bites), with tiger snakes (genus *Notechis*) and taipans (genus *Oxyuranus*) accounting for nearly all other fatalities. Up to 500 cases require snake antivenoms each year, the majority of cases coming from rural areas of Queensland, Western Australia, New South Wales and Victoria, these being the most populated states. The wide availability of snake venom detection kits has allowed specific antivenom to be used more often, rather than polyvalent antivenom, but the latter is still used in nearly 30% of cases, suggesting underutilization of venom detection. The issue of premedication prior to antivenom remains unresolved. Antivenom usage and complication data for 1995 and 1996 will be presented.

Red back spider antivenom is the most commonly used antivenom, with reports of usage being greater than for all other antivenoms combined. It is reported as being therapeutically efficacious in 94% of cases, with a single ampoule being used in 76% of cases, 2 ampoules in 18% of cases, and 3 or more ampoules in 6% of cases. Clinical experience suggests only 20% of red back spider bites require antivenom therapy. It is likely that between 5-10,000 bites occur annually.

S-20Th

ANTIVENOM NEUTRALIZATION OF LOCAL TISSUE DAMAGE INDUCED BY SNAKE VENOMS

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Local tissue damage represents a serious consequence of crotaline and viperine snake envenomations. It encompasses a complex series of alterations, including hemorrhage, edema, myonecrosis and dermonecrosis. Due to its rapid development it is extremely difficult to neutralize by antivenoms if there is a delay in serotherapy. Studies involving the venoms of Central American crotaline snakes indicate that some antivenoms distributed in the region contain antibodies effective in the neutralization of myotoxins, hemorrhagic toxins and edema-forming toxins, although there are products having low antibody titers against these toxins. However, despite the presence of neutralizing antibodies, neutralization of these effects is only partial if antivenom is administered after envenomation. Moreover, neutralization is not complete even if homologous or heterologous antibodies are present in the circulation before venom is injected. Despite differences in their pharmacokinetic profiles, equine whole IgG and $F(ab')_2$ antivenoms show similar efficacy in the neutralization of edema, myonecrosis and hemorrhage induced by *Bothrops asper* venom, suggesting that the production of antivenoms consisting of antibody fragments may not improve the neutralization of these effects. This is apparently due, at least in part, to the fact that microvessel disruption by venom components favors a similar antibody concentration in the affected tissues. Recent advances in the development of neutralizing substances of rapid diffusion, that could be injected locally in the field, may contribute to the neutralization of metalloproteinases and phospholipases A_2. In addition, the rapid administration of antivenoms with high antibody titers against locally-acting toxins is a key element in the treatment of this relevant aspect of snakebite envenomation.

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S-21Th

NEW APPROACHES TO OPTIMIZE ANTIVENOM THERAPY

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The administration of antivenom is the only specific treatment of ophidian envenomings. Although it is widely used and medically accepted, there is no clear agreement about the criteria for evaluating the severity of envenomings or for the administration of antivenom therapy.

Enzyme immunoassays, which allow the precise quantification of the level of toxic antigens in the plasma of envenomed patients are therefore usefull tools to help physicians to decide if immunotherapy is required and to adjust the dose of antivenom to be used. Furthermore, a clear knowledge of the pharmacokinetics of the toxic components as well as that of antivenom antibodies is required for defining accurately the conditions which allow the most efficent treatment: the route of antivenom administration, the type of antibodies (Fab versus Fab'2), the delay of administration...

In order to contribute to the optimization of antivenom therapy, we performed these studies in the case of envenomings following viper bites in France. An ELISA was developed, in parallel with a clinical grading scale based on the earliest chracteristic signs of envenoming. A good correlation was observed between venom levels in the blood of envenomed patients and clinical symptoms. Pharmacokinetic parameters of *Vipera aspis* venom were determined after i.v. and i.m. injections of venom to rabbits. The volume of distribution of the venom was shown to be larger than the vascular compartment. The half life of elimination measured after i.m. injection of venom was three times as high as that measured after i.v. injection. This was explained by a complex resorption process, which was rapide during the first 24 hours and occured at a slower rate over the subsequent 72 hours. The absolute bioavailability of the venom was about 65%.

The effect of antivenom was further tested on venom kinetics. It appeared that the detoxification process of antivenom can be explained by redistribution of the venom from the extravascular compartment to the vascular one, where it is sequestered by the antibodies. Intravenous injection was shown to be the most effective route for antivenom administration. This was explained by the differential kinetic parameters of antibodies and venom proteins when both injected by the extravascular route, (Tmax for Fab'2 48 h and venom proteins 2 h), therefore limiting the probability of interaction between antibodies and venom proteins. The effects of early and late immunotherapy were also determined and the relative efficacy of Fab'2 and Fab was tested. It was shown that in case of the detoxification of *Vipera aspis* venom by injecting antibodies by i.v., Fab'2 are more efficient than Fab, due to their differential pharmacokinetic parameters, in comparison to those of venom proteins, whereas it is the opposite when antibodies are injected by i.m.

These experimental studies could constitute a scientific basis for the optimization of the immunological treatment of envenomings following snake bite.

S-22Th

IMMUNOTHERAPY FOR SCORPION ENVENOMING IN BRAZIL

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Our group has used a tripartite approach to treat scorpion envenoming in Brazil: symptomatic measures, support of vital functions and immunotherapy (Campos *et al.*, 1980; Freire-Maia *et al.*, 1994). Recently, a sensitive and specific enzyme-linked immunosorbent assay (ELISA) was described for detection of *Tityus serrulatus* venom antigens (Chávez- Olórtegui *et al.*, 1994). Using this method in rats our group showed that the absorption of venom from subcutaneous tissue is fast, the distribution of venom to tissues is fast and high, there is a great affinity of the venom for tissues and the elimination half-life is slow (Santana *et al.*, 1996). It has been shown that after hospital admission, patients with systemic manifestations caused by *T. serrulatus* sting had higher plasma venom concentrations (15.07 ± 2.67 ng/ml) than those with only local pain at the site of sting (2.14 ± 0.75 ng/ml) (Rezende et al., 1996). The intravenous infusion of 5-30 ml of scorpion antivenom (Fab_2 fragment, protein content 7 g/100 ml, FUNED, Belo Horizonte, Brazil) over 10 min. into 18 patients with systemic manifestations of *T. serrulatus* envenoming cleared the high levels of venom antigens detected in the plasma 1 hour after starting the immunotherapy. High titers of plasma antivenom were detected for at least 24 hours after antivenom therapy. Vomiting and local pain decreased 1 hour after serotherapy and hyperglycaemia was not detected 12 hours later. Finally, cardiorespiratory manifestations other than pulmonary oedema disappeared 6 - 24 hours after immunotherapy (Rezende *et al.*, 1995). The high titers of plasma antivenom might be required to allow neutralisation of venom that is being absorbed from the site of sting into circulation or to neutralise the venom that is bound to the tissues. Based on our tripartite approach of treating scorpion envenoming (N= 3860 patients) the mortality rate was very low (0.28%).

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S-23Th

PHARMACOKINETICS OF ^{125}I-LABELLED IgG, F(ab')$_2$ AND Fab FRACTIONS OF SCORPION AND SNAKE ANTIVENINS: MERITS AND POTENTIAL FOR THERAPEUTIC USE

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Antivenins of high titre were raised in horses against the scorpion *Leiurus quinquestriatus*, the elapid snake *Walterinnesia aegyptia* and the viper snake *Cerastes cerastes* venoms. The immunoglobulin fractions IgG, F(ab')$_2$ and Fab were separated, purified and labelled. A three-compartment open pharmacokinetic model best fitted the data obtained following the i.v. injection of IgG, F(ab')$_2$ and Fab of the three antivenins into either rabbits or mice. The model comprised a central compartment 'blood', a rapidly equilibrating 'shallow' tissue compartment and a slowly equilibrating 'deep' tissue compartment. In rabbits, and irrespective of the antivenin studied, F(ab')$_2$ possessed the fastest disposition rate constants and the shortest distribution half-lives while Fab showed the lowest disposition rate constants and the longest distribution half-lives. The same picture occurred in mice except that the values for Fab were mostly in between those of F(ab')$_2$ and IgG. F(ab')$_2$ also possessed a significantly smaller area under the curve, AUC, and a significantly greater volumes of the central compartment, V_c, shallow tissue compartment, $V_{t\ shallow}$, deep tissue compartment, $V_{t\ deep}$, and elimination rate constant from the central compartment, k_{10}. F(ab')$_2$ was the fastest and Fab the slowest to achieve maximum tissue concentration, Cp_{max}. In the physiologically based pharmacokinetics, PBPK, F(ab')$_2$ had the highest Cp_{max}, the smallest AUC and the shortest t½β in the different tissues; Fab possessed values in between F(ab')$_2$ and IgG. In anaesthetized rats injected with lethal doses of venoms and infused with neutralizing doses of antivenins, rats infused with F(ab')$_2$ completely recovered, those infused with IgG partially rescued and none of the rats infused with Fab survived. It is concluded that F(ab')$_2$ possessed pharmacokinetic characteristics that render it most suitable for use in serotherapy of scorpion and snake envenoming.

S-24Th

MAPPING THE PORE OF THE T-LYMPHOCYTE POTASSIUM CHANNEL WITH SCORPION AND SEA-ANEMONE TOXINS: FROM TOXIN TO IMMUNOSUPPRESSANT

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The voltage-gated potassium (Kv) channel in T-cells, Kv1.3, regulates the T-lymphocyte activation response. Blockers of this channel, including several high-affinity peptide toxins, inhibit T-cell activation *in-vitro* and suppress delayed type hypersensitivity responses in animal models. The vital role of Kv1.3 during T-cell activation has stimulated a search for potent and selective channel blockers that can be used as models. The vital role of Kv1.3 during T-cell activation has stimulated a search for potent and selective channel blockers that can be used as immunosuppressive agents. Due to its distinct mechanism and restricted tissue distribution, a Kv1.3 blocker would not likely display the toxic side-effects of currently used immunsuppressants such as cyclosporin and FK-506, and therefore may prove useful for treatment of chronic autoimmune diseases as well as transplantation therapy. Delineation of spatial organization of the residues in the pore-region of Kv1.3, the primary target for peptide toxins, would directly aid in the design of novel therapeutic agents. We have determined the architecture of the P-region of Kv1.3 using four structurally-defined, high-affinity scorpion toxins (ChTX, KTX, MgTX and NTX) as molecular calipers. Our analyses reveal the existence of a shallow (~8 angstroms deep), 30-angstroms-wide, saucer-shaped vestibule at the external entrance to the pore. The potassium channel selectivity filter lies in a shallow trough at the center of this external vestibule. Guided by this structural information, we have started a search for selective peptide and non-peptide antagonists of Kv1.3. One such antagonist from sea anemone, a 35 amino acid peptide called ShK toxin, potently blocks Kv1.3 at low pM concentrations. If proven to be a specific inhibitor of Kv1.3, this peptide might have therapeutic use as an immunosuppressant. To determine the selectivity of the ShK toxin, we examined its potency against a panel of six related Kv channels. As expected, Kv1.3 was potently inhibited (IC50 = 12 pM) by ShK toxin with a Hill coefficient close to unity. Kv1.1, a closely related channel expressed in the brain, heart and skeletal muscle, was equally sensitive to the peptide. Another member of the Kv1-family, Kv1.2, was 100-fold less sensitive to inhibition by ShK, while Kv1.5 and Kv3.1 were resistant to the peptide. Obvious concerns regarding ShK toxin's ability to inhibit Kv1.1 with almost the same potency as Kv1.3 necessitated a search for a more selective antagonist of Kv1.3. We have screened several mutants of the ShK toxin in the hope of identifying one that was both a more selective and potent blocker of Kv1.3. Our search has revealed a mutant that retains its ability to potently block Kv1.3, while being >100-fold less effective on the related channels. Using complementary mutagenesis, coupled with mutant cycle analysis, we have mapped the position of the critical peptide residue with relation to the pore residues. Studies are ongoing to determine the immunosuppressive activity of this specific Kv1.3 blocker, both *in-vitro* and *in-vivo*.

S-25Th

^{1}H NMR STRUCTURAL ANALYSIS, USING A NANO-NMR PROBE, OF A NOVEL POTASSIUM CHANNEL BLOCKING TOXIN FROM THE SCORPION *PANDINUS IMPERATOR*.

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Potassium channels toxins act on different types of potassium channels despite their related sequences, 30 to 40 amino acids and three disulfide bridges. Due to the multiplicity of potassium channels types the comprehension of their specific role in modulating cellular function is not trivial. However, the finding of molecules that selectively block a given class of channel is very hepful. The three-dimensional solution structure of a novel peptide, Pi1, purified from the venom of the scorpion *Pandinus imperator* and specific of potassium channels was determined by homonuclear proton 2D NMR methods at 500 MHz from 50 nanomoles of compound. *Pandinus imperator* toxin Pi1 is a voltage-dependent potassium channel specific-peptide capable of blocking the shaker B K^+ channels expressed in Sf9 cells in culture, and causes displacement of binding of labelled noxiustoxin from rat brain synaptosomal membranes. The toxin has only 35 amino acid residues but is stabilized by four disulfide bridges (4-25, 10-30, 14-32 and 20-35) instead of three commonly found in small potassium channel toxins. The dihedral angles and distance restraints obtained from NMR measurements were used in structural calculations in order to determine its solution conformation. It is organized around a short α-helix spanning residues Ser8 to Thr18 and a β-sheet stabilized by two disulfide bridges 10-30, 14-32. The antiparallel β-sheet is composed of two strands extending from Asn22 to Cys32 with a tight turn at Arg28-Met29 in contact with the N-terminal fragment Leu1 to Cys4.

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S-26Th

ION CHANNEL TOXINS: 3D STRUCTURES, CHANNEL-BINDING SURFACES & PROGRESS TOWARDS MINIMISATION

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Polypeptide ion channel toxins are proving to be valuable therapeutic leads in the treatment of a range of conditions. Among their advantages are high potency, good target specificity, high solubility and rapid onset of action. As they are often small proteins cross-linked by several disulfides, they generally also have quite stable structures in solution, which are readily determined using ^{1}H NMR spectroscopy. Once the solution structure has been solved it is possible to map onto that structure the likely channel binding surface, identified initially by alanine scanning, then characterised further by additional residue substitutions. If a model of the ion channel is also available then possible docking interactions of the toxin can be tested by complementary mutagenesis. This information provides the basis for the design of smaller peptidic analogues of the toxin, and eventually of peptidomimetic analogues.

Our work on the solution structure, structure-function relationships and peptidic analogues of two polypeptide toxins, both blockers of voltage-gated ion channels, will be described. The first is ω-conotoxin GVIA, which blocks N-type calcium channels. The structure consists of a small triple-stranded β-sheet and a disulfide knot [1] and an alanine scan has identified residues that contribute to calcium channel binding [2,3]. The second is a potassium channel blocker from a sea anemone, ShK toxin, which is a potent blocker of Kv1.3 potassium channels in T-lymphocytes. The solution structure of ShK toxin consists of two helices and a series of turns, making it quite different from scorpion toxins that interact with the same channel [4]. Key residues for channel binding have been defined using synthetic analogues [5]. For both toxins the structural effects of disulfide bond removal and truncation have been investigated as a first step towards development of a peptidic analogue.

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S-27Th

MAUROTOXIN, A FOUR DISULFIDE BRIDGES SCORPION TOXIN ACTING ON K+ CHANNELS.

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Maurotoxin has been purified to homogeneity by gel filtration and RP-HPLC from the venom of the Tunisian chactoid scorpion *Scorpio maurus* [1]. It is a basic and C-terminal amidated 34 aminoacid residues polypeptide cross linked by four disulfide bridges. From Edman degradation results, only six different pairings between the first six half cystines were possible and one bridge predicted between the last two half-cystines 31 and 34. Modelling based on the structure of charybdotoxin favored 2 different pairing one of which having two bridges in commun with the general motif of scorpion toxins. Synthetic maurotoxin was obtained by the solid phase technique[2]. The half-cystine pairing of s-Maurotoxin was identified by enzymatic cleavage (Cys3-Cys24, Cys9-Cys29, Cys13-Cys19 and Cys31-Cys34) which is in agreement with experimental data obtained with natural Maurotoxin. Both natural and synthetic maurotoxins block the Kv1.1, Kv1.2 and Kv1.3 currents expressed in *Xenopus* oocytes with almost identical IC_{50}. Both of them inhibits, at similar concentrations, the binding to rat brain synaptosomes of ^{125}I-Apamin, a SK_{Ca} channel blocker, and ^{125}I-Kaliotoxin, a K_V channel blocker. As native and synthetic maurotoxins have indistinguishable physical and pharmacological properties, they both very likely adopt the same disulfide pairing which is a unique organization in scorpion toxins. However, this result is different from those obtained with PI_1, a scorpion toxin acting of K+ channels and showing high sequence identity (68 %) with Maurotoxin[3]. A comparative study of the two toxins may be of interest regarding the scorpion toxins structure-activity relationships.

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S-28Th

IDENTIFICATION OF POTENTIAL FUNCTIONAL SITES IN PROTEINS: TOXINS TO THERAPEUTICS

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Protein toxins exhibit their pharmacological effects by binding to specific receptor/acceptor or target proteins. Identification of these interaction sites are useful in developing therapeutic agents. Recently, we developed a simple method to identify potential protein-protein interaction sites directly from the amino acid sequence of a protein, based on the occurrence of proline residues that are commonly found in the flanking segments. Using this method, we have identified the functional sites of proteins, including toxins. For example, we have identified the functional site of calciseptine and related toxins isolated from *Dendroaspis* venoms. These toxins bind specifically to L-type calcium channels and block the calcium currents. According to our method, the functional site of these toxins is located between proline 42 and proline 47. A synthetic peptide, named L-calchin (L-type calcium channel inhibitor), comprising this predicted site, as expected reduces the peak systolic and developed pressure in isolated rat heart without affecting the heart rate or diastolic pressure. Furthermore, L-calchin blocks L-type calcium channel currents in whole-cell patch-clamped rabbit ventricular myocytes. This block is independent of the voltage. Thus we have developed a hypotensive peptide based on the structure of a snake venom toxin. We have also identified protein-protein interaction sites of other protein toxins using this method.

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S-29Fr

CLOSTRIDIUM PERFRINGENS TOXINS

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C. perfringens produces many extracellular toxins. Some are enterotoxic and cause diarrhea (e.g. enterotoxin, ß, ε and ι); some are enzymes (e.g. neuraminidase, α is a phospholipase C, μ is a hyaluronidase and ι is an ADP ribosyl transferase). Some are produced by almost all strains (e.g. α); some are produced by only a few (e.g. enterotoxin). Some may be unique to *C. perfringens* (e.g. ß, ε and enterotoxin), some are known to be produced by other clostridia (e.g. *C. spiroforme* is produces ι). Some *C. perfringens* toxins are also found in unrelated bacterial genera (e.g. a low G+C gene coding for θ, a reversibly oxygen-labile, hemolysin, has been identified in *E. coli*, listeria and other genera). Some toxins are produced during logarithmic growth (e.g. α,ß, ε and ι), at least one (enterotoxin) is coordinately regulated with sporulation. In general, tissue degrading enzymes (e.g. collagenolytic κ) are thought to play a more significant role in extraintestinal gangrenes, though some species of clostridia, e.g. *C. septicum* with no recognized enterotoxins, can cause intestinal gangrene, one symptom of which is diarrhea. Susceptible hosts range from humans, domestic livestock and laboratory, zoo and wild animals. One very typical feature of *C. perfringens* is that colonization by non-resident strains or intestinal overgrowth by native clostridia generally occurs only in animals with abnormal bowel floras. Thus, clostridial diarrheas occur in animals removed from germ-free isolators, at weaning and after other dietary changes, in the immunocompromised and after exposure to antibiotics. *C. perfringens* infection can be prevented by vaccination and avoidance of predisposing factors; they are readily treated with vancomycin and/or metronidazole.

S-30Fr

ENTEROTOXIGENIC *ESCHERICHIA COLI*: VIRULENCE DETERMINANTS AND VACCINES

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Current diarrheagenic *E. coli* vaccines strategies are based primarily on stimulating an immune response to virulence determinants. Among the five types of diarrheagenic *E. coli*, adhesins (also termed colonization factors, CF) have been implicated in the virulence of four [enterotoxigenic *E. coli* (ETEC), enteropathogeneic *E. coli*, enterohemorrhagic *E. coli* (EHEC), and enteroaggregative *E. coli* (EAggEC)], while toxins appear also to be important in four types [ETEC, EHEC, EAggEC, and enteroinvasive *E. coli*].

ETEC diarrhea is common in infants and children from developing regions of the world, with a resultant high morbidity and mortality. Travelers to these areas are also at great risk of acquiring an ETEC infection. CF are the adherence proteins present on the surface of the bacterial cell, allowing initial attachment and colonization, with CF considered strong immunogens as well as protective antigens.

Our efforts have focused on development of a purified ETEC CF based vaccine. To date, both CFA/II (CS1, CS3) and CS6 have been produced and microencapsulated into biodegradable poly(D,L-lactide-co-glycolide)(PLGA) microspheres. Most recently recombinant, fermentation, and scalable bioprocess purification techniques have been utilized. For CS6 the complete four gene operon (approximately 5 kb) was cloned and transformed into HB101. The clone produced CS6 under fermentation conditions. The bacteria were removed by centrifugation and membrane filtration, and the media separated from the CS6 by ultrafiltration. CS6 was further purified by precipitation with ammonium sulfate followed by diafiltration into aqueous sucrose. The antigen was then incorporated into PLGA by a solvent evaporation procedure. After production and microencapsulation of antigen, product was examined by scanning electron microscopy, biochemically analyzed, assayed for microbial load, and animal tested for safety and immunogenicity. Clinical trials of microencapsulated CS6 are currently being planned. Our ultimate goal is to produce a safe, efficacious, inexpensive, multivalent oral ETEC vaccine.

S-31Fr

SHIGELLA VACCINES: PROGRESS AND PROSPECTS

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Shigellosis accounts for approximately 20% of diarrheal disease and for 50% of dysentery occurring children of the developing world. The WHO Global Programme for Vaccines and Immunization has established *Shigella* vaccine development to be among the highest research and priorities for the Expanded Programme on Immunization. Three different immunization approaches are currently being evaluated in clinical trials or in field trials, and each approach has given promising results. (1) A live, genetically attenuated, *S. flexneri* 2a candidate vaccine (SC602) has been successfully tested for safety and immunogenicity in adult, North American volunteers. A subset of the volunteers who had been inoculated with a single dose of SC602 were protected from dysentery and partially protected from diarrhea after challenge with virulent *S. flexneri* 2a. Additional volunteer studies are planned to further assess the safety of SC602. (2) Acid hydrolyzed *S. sonnei* lipopolysaccharide (LPS) has been chemically conjugated to a protein carrier for use as a parenteral vaccine. A single intramuscular injection of this conjugate gave protection against *S. sonnei* diarrhea in field trials conducted by the Israel Defense Force. An *S. dysenteriae* 1 conjugate is currently being tested for safety and immunogenicity in pediatric populations. (3) Meningococcal outer membrane protein vesicles (proteosomes), with non-covalently associated *S. sonnei* LPS, has been constituted as a vaccine that elicits serum and intestinal antibody responses against the *Shigella* LPS when administered intranasally to volunteers. Safety and immunogenicity trials of an *S. flexneri* proteosomes/LPS vaccine are planned.

S-32Fr

ENGINEERED BACTERIAL SUPERANTIGEN VACCINES

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Bacterial superantigens are soluble proteins, expressed by many staphylococcal and streptococcal bacterial strains, that bind to the human MHC class II molecule, HLA-DR, and T-cell antigen receptors. Receptor engagement results in an intense stimulation of the immune system that triggers acute pathological effects. We designed vaccines by site-specific mutagenesis of common receptor-binding motifs present on all bacterial superantigens. Single or multivalent vaccines were used to immunize mice and rhesus monkeys. High, long-lived protective antibody levels were induced in both species, with no adverse health effects noted. Multivalent vaccines stimulated high levels of cross-reactive antibodies that neutralized diverse, structurally related, bacterial toxins. These vaccines will be useful for controlling bacterial superantigen-associated human diseases.

S-33Fr

DEVELOPMENT OF GENETICALLY ENGINEERED VACCINES FOR BOTULINUM NEUROTOXINS

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Synthetic genes encoding non-toxic, carboxyl-terminal regions (~50 kDa) of botulinum toxin (BoNT) serotypes A, B, and E (referred to as fragment C or Hc) were constructed and cloned into the methylotropic yeast, *Pichia pastoris*. Genes specifying BoNTA-, B-, and E(Hc) were expressed as both intracellular and secreted products. Recombinants, expressed intracellularly, yielded products with the expected molecular weight as judged by SDS-PAGE and Western blot (immunoblot) analysis, while secreted products were larger due to glycosylation. Gene products were used to vaccinate mice and were evaluated for their ability to elicit protective antibody titers *in vivo*. Mice given three intramuscular vaccinations with yeast supernatant containing glycosylated BoNTA(Hc) were protected against an intraperitoneal challenge of 10^6 50% mouse lethal doses (MLD_{50}) of serotype A neurotoxin, a result not duplicated by its BoNTB- (Hc) and BoNTE(Hc) counterparts. Vaccinating mice with cytoplasmically produced BoNTA(Hc) and BoNTB(Hc) protected animals from a challenge of 10^6 MLD_{50} of serotype A and B toxin, respectively. Because of the glycosylation problem encounter- ed with secreted BoNT(Hc), our efforts are focused on the purification of products from intracellular expression. The BoNTA(Hc) and BoNTB(Hc) products were purified by using a series of conventional chromatography steps. The purification and characterization of other Hc serotypes is currently in progress.

O-01Mo

Non-RGD Disintegrin-like/Cysteine Rich-Domains of Atrolysin A: Inhibition of Platelet Aggregation by Recombinant Protein and Peptide Antagonists

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Hemorrhagic metalloproteinase toxins from snake venoms which have metalloproteinase, disintegrin-like and cysteine-rich domains typically are significantly more potent than toxins with only a metalloproteinase domain. The disintegrin-like domains of these toxins differ from the disintegrin peptides by the nature of their different disulfide bond structure and, in lieu of the disintegrins' signature RGD (Arg-Gly-Asp) integrin binding sequence, there is a X-X-C-D disulfide-bonded cysteinyl. Due to these apparent differences, the contribution to the overall function of the hemorrhagic metalloproteinases by the disintegrin-like domain has been unclear. We have expressed in insect cells the disintegrin-like/cysteine-rich (DC) domains of the *Crotalus atrox* hemorrhagic metalloproteinase atrolysin A and demonstrated that the recombinant protein (A/DC) can inhibit collagen- and ADP-stimulated platelet aggregation. Using synthetic peptides, we have evidence that the region of the disintegrin-like domain that is positionally analogous to the RGD loop of the disintegrins, is the site responsible for inhibition of platelet aggregation. These synthetic peptides must have the -R-S-E-C-D- cysteinyl residue constrained by participation in a disulfide bond with another cysteinyl residue. The two acidic amino acids adjacent to the middle cysteinyl residue in these peptides are important for biological activity. Additional residues in the 14-member loop region contribute to a lesser degree to the biological activity of the domain. These studies emphasize a functional role for the disintegrin-like domain in toxins and suggest structural possibilities for the design of antagonists of platelet aggregation.

O-02Mo

NEUROTOXIN RECEPTOR SITES ON SODIUM CHANNELS - WHO INTERACTS WITH WHO AND HOW?

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Out of all ionic channels, the voltage-sensitive sodium channels serve as a target for the largest number of ligands, most of them consisting of various natural toxins produced by different animals, plants and microorganisms. The neurotoxins have been used as tools for functional mapping and characterization of sodium channels. At least six distinct classes of sodium channel neurotoxins have been designated on the basis of physiological activity and competitive binding studies. The dramatic physiological effects caused by the toxins binding to distinct receptor sites on the sodium channels may be a result of significant conformational changes induced on the channel protein. For example, binding of alkaloid toxins (such as veratridine and batrachotoxin) at receptor site 2, or brevetoxins, polyether ladder toxins that bind to receptor site 5, alter every aspect of the ion channel function: they shift the voltage dependence of activation to negative membrane potential; inhibit the inactivation process, and alter the ion conductance. These two different groups of lipid-soluble neurotoxins, shown to occupy two distinct receptor sites localized in the hydrophobic core of the sodium channel protein, reveal strong and sometimes, unexpected allosteric interactions with receptor sites of other neurotoxins. Perhaps the most unexpected allosteric interaction have been recently described among three receptor sites: The binding of scorpion α-toxins, that inhibit sodium channel inactivation, has been significantly inhibited by brevetoxin, a sodium channel activator; but increased by veratridine, another activator of sodium channels. Most surprisingly, the inhibition by brevetoxin has been completely reversed by the binding of tetrodotoxin, an universal sodium channel blocker. Equally surprising is the inhibition of delta conotoxin (that inhibit sodium channel inactivation by binding to a distinct receptor site) binding by veratridine. Our most recent study suggest that depolarization-induced conformational changes differentially modulate the effect of each neurotoxin on scorpion toxin binding. Together, these resuls contribute to the clarification of gating processes and the dynamic conformational changes induced on the sodium channel protein by simultaneous occupancy of distinct receptor sites by different neurotoxins. The complex interactions may be understood on the basis of the dynamic voltage dependent conformational and functional changes of sodium channel states.

O-03Mo

A PROBABILISTIC APPROACH TO FIND FUNCTIONALLY SIGNIFICANT LOCI IN SEQUENCES OF PEPTIDIC TOXINS

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All proteins consist of 20 amino-acids. If the amino-acids would combine randomly to make a peptide of length n, one should get 20^n different proteins. Yet, most of these combinations never occur, and the entropy of the set of peptides of size n is $\propto k \cdot \ln(20^n)$ (Boltzmann equation). The reduction in entropy (*negentropy*) results from natural selection of biologically meaningful combinations. An approach to identify which part of the sequence of a peptidic toxin determines its action is to consider several toxins with variable peptidic backbones but same effect. It is customary to align the peptidic sequences with a computer algorithm which produces a tabular output matching the points of greatest homology. We have analyzed Clustal W tables using the multinomial probability distribution function, to calculate the probability that a column C_j is formed randomly: $m(C_j) = n! \cdot \prod \left[\theta_i^{f_i} / f_i!\right]$. Where n is the number of called residues in column j, f_i is the frequency of amino-acid i in the column, and θ_i is the proportion of amino-acid i in the aligned peptides. $m(C_j)$ was compared to the median aligned column probability ($p_{m.shuffled}$) of the same peptides after randomizing their sequences. We have found that, for some peptidic domains, $m(C_j)$ ranges from 10^{-10} to $10^{-5} \times p_{m.shuffled}$. This suggests that such loci are evolutionary selected and contain information (negentropy) relevant to the toxin-receptor interaction.

O-04Mo

Probing for the Involvement of Asparagine 67 in Substrate Recognition of *Trimeresurus flavoviridis* Venom Phospholipase A_2 by Protein Engineering and Molecular Dynamics

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Phospholipase A_2 (PLA_2) catalyzes the hydrolysis of the 2-acyl ester bond of 3-*sn*-phosphoglycerides producing free fatty acids and lysophospholipids. *Trimeresurus flavoviridis* (*Tf*, Habu) snake venom contains a variety of PLA_2 isozymes with different physiological activities. For example, some PLA_2 isozymes have shown strong contractile activity on guinea pig ileum which is relevant to release of arachidonic acid from 2-arachidonoyl-1-stearoyl-L-phosphatidylcholine (ASPC). To elucidate the structure-function relationships on recognition of substrate phospholipids, especially 2-arachidonoyl *sn*-glycero-3-phosphocholine, we studied the functional involvement of residue 67 of Asp-49-PLA_2s. Recombinant mutants of *Tf* Asp-49-PLA_2 in which Asn at position 67 was replaced by Asp, Lys, Ser and Gly were prepared by site-directed mutagenesis and overexpressed in *Escherichia coli*. After renaturation of recombinants, the lipolytic activities of native and mutant enzymes were analyzed in three different assay systems, egg yolk emulsion (micelles) and synthetic phospholipid, ASPC (monomer and micelles). For egg yolk assay, the activity levels of the mutants at position 67, (M8L/N67D)PLA_2 and (M8L/N67K)PLA_2 were comparable to those of native *Tf* PLA_2 and (M8L)PLA_2. On the other hand, the activity levels of (M8L/N67S)PLA_2 and (M8L/N67G)PLA_2 were 17~21 % of native and (M8L)PLA_2. The marked differences were also noted in lipolytic activity toward ASPC. The amount of arachidonate released from ASPC mixed micelles was reduced to 30% for N67D and N67K mutants, 70% for N67G mutant, but no change for N67S mutant. In contrast, for monomeric ASPC, activity was decreased to 40% for N67D and N67G and to 60 % for N67K but no change for N67S. These results suggest that the properties of the side chain of residue 67 exert a significant influence for recognition of 2-arachidonoyl *sn*-glycero-3-phosphocholine. Furthermore, the structural differences between active and lessactive mutants were investigated by molecular dynamics.

O-05Mo

NEWLY IDENTIFIED K^+ CHANNEL TOXINS FROM *TITYUS SP.* AND *CENTRUROIDES SCULPTURATUS* SCORPIONS

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A family of small proteins that act as blockers of a diversity of K^+ channels have been described in different scorpion venoms, mainly from Old World scorpions. In New World scorpions, fewer K^+ channel blockers have been identified, primarily in *Tityus serrulatus* and the genus *Centruroides*. We have discovered new K^+ channel toxins common to genus *Tityus sp. serrulatus, stigmurus,* and *bahiensis* and named them "butantoxins". All three toxins possess an identical amino acid sequence. Butantoxins are unique in having four, rather than three, disulfide bonds and are distinct from previously described Tityustoxins. Sequence homology of butantoxins with the *Leiurus quinquestriatus* K^+ channel blocking protein charybdotoxin (Chtx) is 37% (13/35) and with noxiustoxin (Ntx) from *C. noxius* is 44% (17/39). There is 53% homology (20/36) shared between Chtx and Ntx. Butantoxins from *T. serrulatus* and *T. stigmurus* inhibited proliferation and IL-2 production of antigen-stimulated T-helper cells, but had no effect on IL-2 stimulated responses of the same T-cells; Ntx had the same effect. The Na^+ blocking toxin from *T. serrulatus* called toxin γ had no inhibitory effect on any of these T-cell responses. Other peptide purification studies of *C. sculpturatus* venom Zone 12 revealed two new K^+ channel toxins, CsVK1 which shares near sequence identity with Ntx (37/39) and CsVK2 which is closest to that of Toxin 1 (*Centruroides limpidus limpidus*) (34/38).

O-06Mo

NMR structure of a T epitope-containing cyclic peptide with restricted conformations and increased neutralizing antibody responses against the toxin α from *Naja nigricollis* snake

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We have calculated NMR solution structures of a synthetic cyclic peptide containing the peptide sequence from amino acid 24 to 41 of the *Naja nigricollis* snake toxin α. The cyclic peptide is made by formation of a disulfide bridge between the N-terminal Cys24 and the C-terminal Cys41. Furthermore, Cys24 and Cys41 were replaced by penicillamines (3,3-dimethyl-L-cysteines). This structural variation increases rotational conformation barriers around χ_1, χ_2 and χ_3 torsional angles of the disulfide bridge. This structural variation is associated with an increased neutralizing antibody response against the toxin α [M. Léonetti, J. Cotton, S. Leroy, G. Mourier & A. Ménez, *J. Immunol.* 155 (1995) 210]. The NMR structures were calculated with identical experimental conditions [2nM aqueous solution at pH 5.4 and 3°C] to that of the non-modified peptide with regular Cys [P. Cuniasse, A. Thomas, J. Smith, H. Lam Thanh, M. Léonetti & A. Ménez, *Biochemistry* 34 (1995) 12782]. NMR-derived models indicate that the peptide is spread in a more restricted conformational space with backbone atomic root-mean-square difference of 1.58 ± 0.62 Å to the average from residue 24 to 41 and 0.76 ± 0.20 Å from residue 28 to 36 that include a turn similar to the native toxin α. Possible relationships with the increased antibody responses will be presented.

O-07Mo

CHEMICAL REACTIVITY OF CHARYBDOTOXIN: AN ELECTRONIC STRUCTURE BASED POINT OF VIEW.

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A detailed analysis of the electronic structure of a potassium channel blocker [Charybdotoxin (ChTX)] is presented within the context of the ab-initio total energy density functional pseudopotential method(1) (TEPC). We obtain local softness(2) and the electrostatic potential for the molecule. With these two properties we perform an analysis of the reactivity of ChTX. Our approach is oriented towards a comparison with site directed mutagenesis experiments related to ChTX-Channel interaction. As it is expected, the positive values of the electrostatic potential in ChTX match with the positions of the charged aminoacids. Local charge transfer effects from ChTX to the Channel, does not show any particular feature related to the crucial aminoacids. In contrast, local charge transfer towards ChTX capability, has its highest values located in positions that correspond to the experimentally determined(3) crucial aminoacids. Thus, the combination of the electrostatic potential and local softness can be used to give a complementary picture (electrostatic and charge transfer) of the complex interaction between macromolecules. The results obtained show that by using TEPC methodologies, it is feasible the ab-initio study of regional reactivity in polypeptides on an aminoacid by aminoacid basis if reliable geometries are available.

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O-08Mo

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Identification and functional study of an acetylcholine receptor-mimicking antibody/short-chain toxin complex as revealed by molecular modelling and mutational analysis.

Mα2-3 is a monoclonal antibody that specifically recognizes curaremimetic short-chain toxins from snake (1) which are potent antagonists of the nicotinic acetylcholine receptor (AchR). Also, we recently demonstrated that Mα2-3 partially mimics AchR, since they both share a number of functional, structural and antigenic properties (2). Therefore, we decided to tentatively shed light, indirectly, as to the toxin binding site on the AchRs by studying the functional architecture of the paratope of Mα2-3. To reach that goal, we (i) predicted by molecular modelling its three-dimensional structure (3); (ii) designed a convenient bacterial expression system allowing the production of functional scFv fragments of Mα2-3 fused to alkaline phosphatase as reporter enzyme; (iii) developed a sensitive ELISA to rapidly evaluate the affinity of Mα2-3/toxin complexes; (iv) submitted 80% of exposed to solvent residues to individual or combined mutations (4). The functional architecture of the paratopic area of Mα2-3 resulting from the mutational analysis will be shown and discussed. In addition, double mutations done on the antibody and toxin, allowed us first to identify a pair of functional residues in a close proximity to each other upon complex formation and, second to propose an experimentally-based model of an antibody/toxin complex, without NMR and RX data.

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O-09Mo

The NMR Studies of Cardiotoxins from Taiwan Cobra (*Naja naja atra*): Structure, Dynamics, Folding and Interaction.

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Cardiotoxins (CTXs) are the snake venom proteins isolated form the Taiwan Cobra. They contain 60 amino acid residues and four disulfide bonds. The NMR solution structures of CTXs were determined with hybrid distance geometry-dynamical simulated annealing technique. It shows CTX to be three-finger proteins which contains an anti-parallel double and triple strands, with three loops. When CTXs was dissolved in 80% TFE solution, a molten globule-like intermediate was found. The NOE data show that secondary structure is intact and tertiary structure is lost. To characterize the intramolecular dynamics of CTXs, the ^{13}C spin-lattice, spin-spin relaxation time and $\{^1H\}^{13}C$ NOE enhancements have been measured for the backbone C_α carbons by two-dimensional proton-detected heteronuclear NMR spectroscopy on 14.1 Tesla instrument at natural abundance. The relaxation parameters have been analyzed using a model free formalism that depends on the overall rotational correlation time (τ_m), the order parameter (S^2) and internal correlation time (τ_e) for C_α carbons.

O-10Mo

Modelling of Acanthoxin, a PLA2 from the venom of the Australian death adder.

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Phospholipase A2 (PLA2) enzymes are one of the main toxic components present in venom of Australian snakes. Recently, a PLA2 enzyme (acanthoxin) was purified from the venom of the Australian death adder (*Acanthophis antarcticus*.) This enzyme has now been fully sequenced and we present here the results of the three-dimensional modelling of the toxin and its comparison to known X-ray structures. The program MODELLER3 was used to build the model using notexin as the template due to its high homology (~70%) and known crystal structure. The model was then refined using X-PLOR. The model of acanthoxin was then compared to known crystal structures of bovine and porcine pancreatic, human type II non-pancreatic, notexin and *Crotalus atrox* PLA2 enzymes. Additionally, GRASP surfaces (electrostatic surface potential) for the model were compared to surfaces for these PLA2 enzymes. The model was found to have a high degree of structural similarity to the other PLA2 enzymes containing the typical α-helix and β-sheet arrangement of PLA2 enzymes. However, GRASP surfaces revealed notable differences in charge distribution between the toxic and non-toxic PLA2 enzymes. The most striking differences were seen on the non-catalytic surface of the molecule where the toxic PLA2s contained negative residues not present on the non-toxic PLA2s. It is possible that this surface is involved in the interaction between toxic PLA2s and the presynaptic receptor molecules.

O-11Mo

CROTOXIN SUBUNIT CROSS-LINKING WITH A CHEMICALLY CLEAVABLE REAGENT

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Our laboratory has had a long interest in determining whether the heterodimeric rattlesnake neurotoxins, such as crotoxin, are required to dissociate to express their toxicity. We used chemically cleavable protein cross-linkers to probe this problem, concentrating on ethylene glycol *bis*[succinimidylsuccinate] or EGS, which is a homobifunctional NHS-ester cross-linker which can be cleaved by incubation with hydroxylamine. Our strategy was to react the isolated acidic subunit of crotoxin with excess cross-linker. Following acidification, excess cross-linker and 'activated acidic subunit' was separated by gel filtration on Sephadex G25. Recovered 'activated acidic subunit' was combined with stoichiometric amounts of crotoxin basic subunit and reacted at pH ≈8. Acidic subunit 'activation' was indicated when attempts to cross-link the subunits of intact toxin were only moderately successful. EGS reacts with nucleophiles (ε-amino groups of Lys) and the basic subunit has a 4 to 1 ratio of Lys groups relative to the acidic subunit. Basic subunit therefore reacts preferentially, giving an excessive number of one-half reacted EGS cross-linkers. We optimized reaction conditions and recovered the cross-linked toxin by gel filtration on Superdex-75 in the presence of 6M guanidine-HCl. In the absence of reducing agent the product generates a new prominent band on SDS-PAGE with a mol. wt. ≈25kD. Treatment with hydroxylamine results in reappearance of proteins migrating as expected for crotoxin subunits. Mass spectral analyses of recovered cross-linked crotoxin shows the presence of a 24kD component. Cross-linked crotoxin and hydroxylamine-treated cross-linked crotoxin both retain partial PLA_2 activity toward L-α-phosphatidylcholine, but were not lethal to mice at 0.33 μg/g (i.v.). Supported in-part by USAMRDC under Grant DAMD 17-92-J-2017. We thank Dr. Allan Bieber of Arizona State University for performing the mass spectral analyses.

O-12Mo

IDENTIFICATION OF ALLERGENIC COMPONENTS IN SNAKE VENOM BY HUMAN ANTIVENOM IgE

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Hypersensitivity to snake venom due to repeated contact, bites or sensitisation has been described (Parrish, H. M and Pollard, C. B..(1959), Am. J. Med. Sci. 237:277-286). However, few have identified the allergenic agent and for bothropic venoms no reports could be found in the literature. Using human serum from individuals which were allergic to snake venom, nonallergic or had a history of snake bites, we studied the venoms of *B. jararaca, B. jararacussu, B. alternatus, B. atrox, B. neuwiedi, B. moojeni* and *Crotalus sp.* for IgE reactivity. In Elisa studies significant IgE reactivity was observed in the venoms of *B. jararaca, B. jararacussu, B. neuwiedi* and *Crotalus sp.* against the sera of the allergic individuals. IgG reactivity was observed for all venoms studied against the sera of allergic and snakebitten individuals. The IgE reactivity was corroborated in Western blot analyses and allergenic components with molecular masses ranging from 18.3 kDa to 30 kDa were identified for the venoms tested. For the *B. jararuca* venom IgE reactive bands were observed at approximately 18.3, 22.5 and 30.0 kDa.; for *B. jararacussu*, a single band of approx. 23.9 kDa.; for *B. neuwiedi*, at 24.5 and 22.3 kDa. and for the *Crotalus sp.* venom at approx. 18.9, 20.0 and 21.0 kDa. The pattern of IgE reactivity amongst the allergic individuals may suggest the existence of species-specific and species-shared components as well as a genetic variation of their immune response. The controls sera showed no IgE nor IgG reactivity. A fraction of the *B. jararacussu* venom with IgE reactivity, obtained from a DEAE-Sepharose column was further fractionated on a Sephadex G-100 and on an HPLC system on a reverse phase column. Two fractions were obtained with molecular masses of approximately 23.9 and 34.0 kDa.

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O-13Mo

ISOLATION OF A PROCOAGULANT PROTEIN FROM BRISTLES OF *Lonomia obliqua* CATERPILLARS

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Skin contact with *Lonomia* caterpillars causes hemostatic disturbances. This accident was first described in Venezuela (*L. achelous*) and a very high incidence has been reported during the last eight years in South Brazil (*L. obliqua*). Bristles extracts of *L. obliqua* caterpillars show strong procoagulant activity on human plasma *in vitro* which probably accounts for the *in vivo* effect. Therefore, the purification and characterization of the active component was undertaken. Extracts were prepared by macerating bristles in cold phosphate-buffered saline and submitted to Sephadex G75 gel filtration in 50mM TRIS-HCl buffer pH 8.0. Activity of eluted fractions was monitored by the recalcification clotting time of human plasma. After dialysis and concentration the pool of active fractions was submitted to ion exchange chromatography on DEAE-Sephadex A50. The column was equilibrated with the same buffer and eluted step-wise with NaCl (80-500mM). The SDS-PAGE analysis demonstrated a protein of 20kDa with procoagulant activity on human fibrinogen added of factor II in the presence of calcium.It was able to activate factors II and X in assays on specific chromogenic substrates but no amidolytic activity on these substrates was displayed. Considering the potential application of this protein as an anticoagulant a cDNA library with a titer of 10^8 pfu/µg DNA was constructed in a lambda Zap vector (Stratagen) from 7.5µg of mRNA extracted from caterpillar bristles. (Supported by FAPESP and Fundação Butantan).

O-14Mo

COMPONENTS AND CHARACTERISTICS OF GRAMMASTOLA CALA VENOM, THE BEAUTIFUL TARANTULA OF CHILI

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Specimens of Grammastola cala, The Beautiful Tarantula of Chili, have been maintained in The Entomology Insectary at OSU for venom acquisition. Approximately 200 microliters of venom are obtained by electrostimulation from 60 spiders monthly. This specie's venom contains about 20 percent solids. The venom is clear, colorless with a pH of 5.5 and is toxic to mice, lizards, crickets and roaches. Pooled venom samples of 200 to 500 microliters have been fractionated on Sephadex G-50 and G-25 and the major components found in North and Central American Tarantulas are also present. The protein hyaluronidase and peptide lethal toxins are present at lower concentrations than other venoms studied while the paralytic acyl polyamines are present at higher concentrations. The nucleotide fraction cannot be resolved from the acyl polyamine fraction on Sephadex -G50 so a second column of G-25 is required. The citrate content of this venom is about 12% of the dried solids by a coupled enzyme assay. Toxin structures will be presented.

O-15Mo

MALDI-TOF MS FINGERPRINTING OF TARANTULA VENOMS: Taxonomic and pharmacological applications.

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Tarantula venoms are a potentially rich source of novel peptide ligands but have been barely investigated to date, although the large size and availability of these spiders through the pet trade makes them attractive for the search of novel neurotoxins.
However, problems of species identification and taxonomy remain, and are difficult to solve through conventional methods. In particular, the genus *Brachypelma* comprises morphologically similar species and animals raised in captivity may not be properly identified. HPLC and MALDI-TOF mass spectrometry can be used to fingerprint the venoms and accurately discriminate between species. We have obtained venoms from 6 Brachypelma species and compared their HPLC and MS profiles. Although HPLC profiles are almost identical, MS fingerprinting of the major peptide components permitted a clear distinction and enabled us to correctly identify a previously undetermined specimen, as *B. smithi*. Peptide toxins have molecular weights ranging from ca. 3500 to 8000. In addition, these results can be used for pharmacological investigation of the venoms. The previously identified toxin BsTX1 from *B. smithi* was localized and analysis of other species show the presence of putative homologues in all other *Brachypelma* venoms, an interesting finding for structure-activity studies of natural toxins. As toxicity symptoms displayed by mice upon intracerebroventricular injection of the venoms were identical, a series of homologous peptide toxins can be expected and their isolation will be facilitated by the prior knowledge of MW distribution in the venom fractions.

O-16Mo

ALL THREE FREE- SWIMMING STAGES OF *Linuche unguiculata* (THIMBLE JELLYFISH) CAN CAUSE SEABATHER'S ERUPTION

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Seabather's eruption (SBE) is a highly pruritic eruption in swimmers and divers in the marine waters of the Florida and Caribbean area. Its cause has been widely attributed to various organisms and more recently to the larva of the scyphomedusa *Linuche unguiculata*. In the Mexican Caribbean, particularly in the Cancun and Cozumel area, we have been noting the incidence of SBE during the season of high touristic activity, and is of public health concern, which runs from February through June. This corresponds to the time of the life cycle in which the three swimming stages of *Linuche* can be sequentially observed; the ephyra, the medusa (adult) and the larval (planula). In contrasts to literature reports, we have concluded that all three stages can cause SBE and our findings are supported by: (1) observation of the particular stage by marine biology investigators, graduate students in marine biology and professional SCUBA divers coinciding with stinging outbreaks and confirmed by planktonic tows. (2) microscopic observation of discharged nematocysts in skin scrappings and (3) serological evaluation of individuals who had developed SBE after contact with a particular swimming stage. We have also been evaluating, in children and adults, the best course of treatment using antihistaminic and anti-inflammatory agents. In this study, two treatment regiments were used: (1) the combination of isopentyl pheniramine and lidocaine and (2) dermatovate and ibuprofen. In both groups, relief of the intense pruritic and duration of SBE was noted. Haemolytic activity, using sheep red blood cells, has been observed in the crude extract of adult *L. unguiculata* and chromatography conditions are being developed for its purification.

O-17Mo

A COMPARISON OF AUSTRALIAN AND JAPANESE REDBACK SPIDER VENOM

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Upon the first discovery in 1995 of the Redback or Black widow spider (*Latrodectus mactans)* in Osaka, Japan, the Australian Venom Research Unit was invited to assist the Osaka public health authorities investigate the problem. We report here a comparison of the venom of spiders from diverse regions of Australia and from Osaka, Japan. This study was undertaken to (i) determine whether the Australian Redback spider antivenom would neutralise the Japanese spider venom and (ii) assess Redback spider venom protein variation within Australia. Firstly we compared venom samples extracted from Redback spiders sent frozen from Osaka to Melbourne with Redback spiders from rural Victoria. Morphologically the Osaka specimens resembled *Latrodectus mactans* hasselti, the Australian Redback spider. The venom yield from these spiders was similar to that of the Victorian Redback venom gland (40µg per gland). We believe that we have identified α-latrotoxin in the Osaka venom gland extracts that closely resembles the Victorian Redback spider venom standard using SDS-PAGE. Indeed western blotting suggested that the venom from the Japanese spiders would be effectively neutralised by the Commonwealth Serum Laboratories Redback spider antivenom. *In vivo* lethal dose (LD_{50}) and effective dose (ED_{50}) studies will be reported. Venom from Queensland, Victorian, South Australian and Western Australian Redback spiders were compared by SDS-PAGE. No regional variation was observed amongst the major venom proteins.

O-18Mo

COMPARATIVE STUDIES ON VENOM EXTRACTS FROM THREE JELLYFISH: THE IRUKANDJI *(CARUKIA BARNESI)*, THE BOX JELLYFISH (*CHIRONEX FLECKERI* SOUTHCOTT) AND THE BLUBBER (*CATOSTYLUS MOSAICUS*).

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The objectives of this study were to isolate crude venom from the Box Jellyfish (*Chironex fleckeri*), the Irukandji jellyfish (*Carukia barnesi*) and the Blubber jellyfish (*Catostylus mosaicus*) and to undertake comparative studies of their venom using SDS PAGE. Crude venom from Box Jellyfish isolated nematocysts, and soluble fractions from whole Irukandji bells and *Catostylus* oral lobes, were obtained in quantity by a simple method involving 10-15 minutes of chilled glass mortar and pestle grinding in phosphate buffered saline. The use of more sophisticated techniques including homogenisation, freeze thawing and sonication were not advantageous. Preliminary study of the jellyfish crude venoms and soluble fractions by SDS-PAGE was undertaken and extraction procedures were compared. Box Jellyfish crude venom and Box Jellyfish milked venom SDS PAGE protein profiles were compared. Irukandji and *Catostylus* soluble fractions SDS-PAGE protein profiles have been established for the first time. Box Jellyfish antivenom binds to the crude venom of Box Jellyfish, Irukandji jellyfish and *Catostylus* jellyfish by Western Blot. Lethal activity in the Irukandji jellyfish soluble fraction was demonstrated in mice by intravenous LD_{50}. Partial breakdown of the Box Jellyfish crude venom protein of MW 40,000 was observed with a corresponding increase in protein at MW 17,000 after freeze thawing. The Box Jellyfish venom component of approximate MW 40,000, (as estimated by SDS PAGE) may be present as a dimer of subunits of approximate MW 17,000. Immunisation of rabbits with the Irukandji venom lethal fraction is the next step towards antivenom production.

O-19Mo

DOMOIC ACID (ASP) IMPLICATED IN MARINE ANIMALS CASUALTIES IN THE GULF OF CALIFORNIA, MEXICO.

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ABSTRACT We have demonstrated the presence of domoic acid as the causative agent of the mass killing of brown pelicans (*Pelecanus occidentalis*) in the tip of the Baja California Peninsula, Mexico in 1996. We then presumed that it was an isolated event. Now, during January-February 1997, we have witnessed a new event of mass toxicity and mortality of marine organisms: 766 exemplars of the common loon (*Gavia immer*) and 183 sea mammals, belonging to 5 different species, were found floating on the sea or stranded in the beaches of the Gulf of California. Microscopic analyses of gut content showed the presence of domoic acid and remainers of *Pseudonitzchia* (frustules) in common dolphins (*Delphinus capensis*) and sardine fish (*Sardinops sagax*) found inside some of the dolphin stomachs. Furthermore, HPLC analyses of mammal tissue extracts confirmed the presence of domoic acid and some isomers within them. Hystopathological observations showed lesions resembling an acute toxic syndrome in the brain, heart, liver and lungs of some exemplars. Surface temperature data from the Peninsula west coast, shows an evident cooling trend since winter 1992. This may allow cold water species such as *Pseudonitzchia* to travel southwards along the coast, reaching the tip of the peninsula and continued northwards inside the Gulf of California by the winter currents. When these organisms find a front of strong upwelling condition, a local bloom, sometimes with poisoning consequences for marine animals may occur.

Key Words: Domoic Acid, ASP, *Pseudonitzchia sp.*, marine mammals, birds

O-20Mo

Toxicity of Japanese newt, *Cynops pyrrhogaster* of western Kyushu region, Japan

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Newts are known to be toxic. Each toxin of California and "Shiriken" newt toxin was already characterized to be tetrodotoxin (TTX) as main component. Although Japanese newt "akahara" *C. pyrrhogaster* was also reported to be toxic, the properties of the toxin remain uncertain. This report treats with biological and chemical aspect on Japanese newt toxin.

Nearly 400 specimens of Japanese newt were collected from 8 areas at Nagasaki Prefecture and 4 ones at Saga Pref., in the district of western Kyushu, Japan through July 1996 to May 1997. The local, seasonal, anatomical and growth dependent variation of toxicity in the newt was investigated: some of individuals collected from each area were separated into skin ,muscle, viscera and gonad. Whole eggs and larvae were also supplied. Resultant parts were submitted to the mouse assay and HPLC analysis. Some of alive individuals were examined for resistibility against TTX.

The toxin extracted from toxic skin with muscle was purified by several kinds of column chromatographies using activated charcoal, Bio-Gel P-2 and Bio-Rex 70. The chemical properties of the purified toxin were investigated from ^{1}H-NMR and UV absorption spectrum.

Although non-toxic specimen was not found in the areas investigated ,big individual and local variation of toxicity was recognized. In the anatomical toxicity, skin is generally the highest (maximal score: 335MU/g), followed by muscle ,ovary and liver. The liver shows rather low or no toxicity score (<5-10MU/g) and male gonad is non-toxic. The toxicity of the male is generally higher than those of the female. Newts showed high resistibility (more than 1200MU/20g) against TTX, as TTX bearing puffer is so.

The newt toxin was concluded to be TTX as the main component, from the results of its ^{1}H-NMR spectrum and UV absorption one of its alkaline degradation product, in comparison with those of TTX.

O-21Tu

F(ab')2 SNAKE ANTIVENOM (EQUINE) USE IN MARTINIQUE: EFFICACY AND SIDE-EFFECTS

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A new, specific F(ab')2 antivenom (FAV) was developed to treat patients envenomed by *Bothrops lanceolatus*. The goal of venom neutralization is to prevent the severe thromboses (mainly cerebral, but also myocardial infarctions and pulmonary embolisms) observed in 20 to 40% of untreated patients. A clinical study was designed to survey the continuing efficacy of the first generation FAV or of a new, purified, heat-treated version called Bothrofav™, as well as to record any potential side-effects.

All envenomed patients, aged 6 to 82 years, were treated upon hospital admission according to clinical severity by intravenous infusion of 20 to 60 mL of FAV. From April 1993 to January 1997, 45 patients were treated with the first generation product and 21 patients with Bothrofav™. Four of 66 patients developed thrombotic complications before treatment; one of them died. Time from bite to "Favotherapy" ranged from 20 minutes to 60 hours (mean ± sd, 4.5 ± 9.3 hours), and was < 6 hours in 85% of patients (56/66). The mean dose of FAV administered was 31 ± 17 mL, and the mean infusion time was 172 ± 91 minutes.

Five of 66 (7.6%) patients developed reactions to FAV. Two severe reactions occurred, both in the patients treated with the first generation product: one developed hypotension and dyspnea during infusion of FAV, which responded quickly to epinephrine; and another presented with serum sickness (day 9) that required a 3-week steroid treatment. No such severe reactions were reported in patients given Bothrofav™. Overall, 17% of patients (11/66) needed surgery-two fasciotomies and nine incisions of local abscess or debridement of skin necrosis-within 8 hours to 10 days after being bitten. The mean length of hospitalization was 5 ± 6 days for treated patients and 16 ± 34 days for untreated patients. Sixty-five patients recovered (98.5%). No thrombotic complications were observed after either FAV administration.

Early infusion of FAV is an effective and well-tolerated treatment of *Bothrops lanceolatus* envenomation.

O-22Tu

PRACTICAL ASSESSMENT OF ANTIVENOMS

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The amount of antivenom given to a systemically envenomed human depends, among other things, on its ability to permanently reverse the clinical signs of envenoming and on the experience of the individual clinician in assessing this reversal. The processes in the development and assessment of a new or existing antivenom therefore rely on initial experimental followed by clinical and finally pharmacokinetic studies. Experimental studies usually start with an *in vivo* assessment of the neutralisation of the overall lethal effects of a venom (ED_{50} assays) together with, in the case of most viperine venoms, intradermal skin tests for estimation of haemorrhagic and necrotising venom activities. Such *in vivo* studies are unpopular as they cause considerable suffering to the experimental animals used. We are currently investigating the use of fertile hens' eggs as a means of replacing such rodent assay systems. Other *in vitro* studies are also used to assess neutralisation of venom coagulant, anticoagulant and neurotoxic activities. Providing the experimental and ancillary studies (eg sterility, pyrogenicity etc.) yield satisfactory results, the antivenom can then be submitted to clinical trials in which the product is assessed by its ability to reverse the systemic effects of the venom in human victims of snakebite usually in randomised, blinded comparison with another antivenom. During such clinical trials enzyme immunoassay can be used in order to obtain an objective measurement of the ability of the antivenom to clear the venom rapidly from the circulation. Using such a combination of experimental, clinical and immunological assay systems, it is therefore possible to obtain an accurate assessment of the efficacy of antivenoms.

O-23Tu

A CRITICAL REAPPRAISAL OF ANTIVENOM POTENCY ASSAYS

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The use of anaesthetized rats injected with lethal doses of venoms and infused, after various time intervals, with neutralizing doses of antivenoms served as a good model that helped establishment of treatment protocols with serotherapy. It was found that rats injected with single or double lethal doses of different venoms could be rescued by the infusion of neutralizing doses of antivenoms calculated from the *in vitro* mouse assays. Rats injected with multiple venom lethal doses, however, could not be rescued by the infusion of multiple or larger doses of antivenoms. Rats injected with multiple lethal doses of pure toxins and infused with neutralizing doses of specific antivnoms could be rescued. This suggested that venoms may contain low concentrations of toxic components that are poorly antigenic and would not raise antibodies when using crude venoms as antigens. These would accumulate and kill the animals when larger doses of venoms are used. Comparative assays were made for refined horse serum antivenoms raised against *L. quinquestriatus, W. aegyptia, N. haje, C. cerastes, E. carinatus* and *B. arietans* venoms using either a fixed dose of antivenom and increasing venom concentrations or a fixed venom dose (5 LD_{50}/mouse) and serial dilutions of antivenoms. The potency when diluting the antivenoms was 1.6-4 fold that obtained by increasing venom concentrations. The results of the rescue experiments in rats correlated more favourably with the fixed dose antivenom/increasing venom concentrations mouse assays. It is concluded that in the potency assays of antivenoms, animals should be challenged with high doses of venoms to ascertain the neutralizing abilities of antivenom against possible non-neutralizable toxic components.

O-24Tu

NEUTRALIZING HUMAN ANTI-CROTOXIN scFV ISOLATED FROM A NON-IMMUNIZED PHAGE LIBRARY

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The display of repertoires of antibody fragments on the surface of filamentous bacteriophages offers a new way of making antibodies with predefined binding specificities. Here we explored the use of this technology to make human antibodies with biological properties. Antibodies fragments specific to crotoxin, the main toxic component of the venom of the South-American rattlesnake *Crotalus durissus terrificus*, were isolated from a repertoire of more than 10^{10} single-chain Fv (scFv) made *in vitro* from human V gene segments (*Nature Biotechnology*, 14: 309-314). ScFvs are recombinant antibody fragments consisting only of the variable light chain (VL) and variable heavy chain (VH) domains covalently connected to one another by a polypeptide linker. V-genes were isolated from 43 non-immunized donors using PCR primers corresponding to all known VH, Vκ and Vλ gene families. ScFv fragments were cloned in a phagemid vector, enabling both phage displayed and soluble scFv to be produced without subcloning. The crotoxin molecule is composed of two non covalently linked subunits: a basic and weakly toxic phospholipase A_2 (PLA_2) called component B (CB) and an acidic, nonenzymatic and nontoxic subunit called component A (CA). This one is able to increase the toxicity as well as the specificity of action of CB. After three rounds of panning, fourteen phage-svFv were isolated and three of them have been extensively studied (scFv1, scFv6 and scFv8). They recognize distinct epitopes on crotoxin. Clone 6 is specific of the basic subunit, CB, and clones 1 and 8 recognize CB and CA. Clone 1 is able to increase, *in vitro*, the enzymatic activity of crotoxin suggesting that this clone might dissociate the crotoxin complex. Moreover, a svFv1 dose-dependent protection was observed *in vivo* suggesting that this clone has a neutralizing activity. The other clones are being tested regarding their neutralizing ability of crotoxin.

Supported by European Community, FAPESP, Fundação Butantan.

O-25Tu

POLYSPECIFIC OVINE FAB FOR THE TREATMENT OF CROTALID SNAKEBITE IN THE UNITED STATES.

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Over the past 4 years, a new Fab antivenom for Crotalid snakebite in North America has been tested in humans. METHODS: Two trials have been completed: 1) a pilot study of 11 patients treated with single dose Fab; 2) a randomized trial of 30 patients comparing 2 dosing regimens of Fab. Both were multicenter trials and enrolled patients with worsening crotalid snakebite. In trial 1, each patient received a single Fab dose of 4-8 vials while in trial 2 each patient received 6-12 vials to establish "initial control" and was then randomized to receive an additional 2 vials every six hours for 3 doses (SCHEDULED Group) or to receive additional Fab only if the investigator decided it was clinically needed (PRN Group). A severity score (SS) was used to assess bite severity over the first 12 hours. Efficacy was defined in both trials as no increase in the SS. Adverse events were assessed using formal evaluations throughout hospitalization and for 2 weeks after discharge. RESULTS: All patients had a beneficial response based on SS. Early reactions occurred in 14.6%; serum sickness occurred in 14.6%. 1 case developed bronchspasm. Of 15 patients in the PRN group, 13 (73.3%) required additional Fab administration after the initial dose ($p < 0.0001$). Re-emergence of coagulopathy was noted in 19 of 41 patients (46.3%). Analysis of a pre-existing database of crotalid snakebite revealed that re-emergence of coagulation abnormality with Antivenin (Crotalidae) Polyvalent [Wyeth] also occurred commonly. CONCLUSION: Polyspecific ovine Fab is safe and effective in the treatment of North American crotalid snakebite. Repeated antivenom dosing appears preferable to single dose therapy. In addition, re-emergence of coagulation abnormalities occurs regularly after bites of North American rattlesnakes.

O-26Tu

THE SIMULTANEOUS INJECTION OF NATIVE AND TOXOIDED BOTHROPIC VENOM ABOLISHES THE APPEARANCE OF ACUTE AND CHRONIC LESIONS INDUCED BY THE NATIVE VENOM.

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The injection of subletal doses of *Bothrops jararaca* venom is followed by acute local lesions and chronic systemic lesions (*Toxicon* 28, 171-9: 1990; Dtsch tierärztl Wschr 99, 143-5: 1992). The larger the dose injected, the more intense and extensive are the lesions. This follows the general finding of biological response to antigens inoculated. Local lesions were mostly tissue necrosis, both cutaneous or myonecrosis; the most conspicuous of the chronic lesions was an infiltration around the white pulp of the spleen. The substance had staining properties of an amiloid material. We observed that venoms haptenized to the point of abolition of letal capacity, were unable to cause any pathological alteration in mice and rabbits, but induced an immunological response. Then, what would happen if both forms of the venom were applied simultaneously? Groups of mice were injected with the native (NAT) venom, and at the same occasion, in the contralateral side, they received the toxoided (ATX) venom by stepwise iodination. The doses of ATX venom were 1, 3 or 6 times that of the NAT venom. The schedule was 10 applications spaced by 4 days. Histological findings were: group of 1 NAT / 1 ATX, attenuation of tissue lesions in most animals; group of 1 NAT / 3 ATX an accentuated decrease with 40% lesion-free mice; group of 1 Nat / 6 ATX, almost disappearing alterations, with more than 80% lesion-free mice. This suggests that abolition of lesions was related to the amount of toxoided venom inoculated. The result has extended importance because it can be applied in other similar situations to avoid lesions caused by antigens, even in autoimmunity aggressions.

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O-27Tu

PURIFICATION, AMINOACID SEQUENCE, MOLECULAR CLONING AND EXPRESSION OF NON-TOXIC PROTEIN FROM *TITYUS SERRULATUS* SCORPION THAT INDUCES THE PRODUCTION OF ANTIVENOMS

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Fractionation of the venom from the scorpion *Tityus serrulatus* by combining gel filtration, ion exchange and reverse phase chromatographic steps we were able to purify to homogeneity a non toxic protein (TsNTxP). TsNTxP is a basic protein (pI 8.96) composed of 63 amino acids residues, and has a calculated molecular weight of 6.759 kDa. A high degree of structural homology is found when TsNTxP is compared to toxins isolated from the *Tityus serrulatus* venom. Subcutaneous injection of TsNTxP in mice, rabbits or horses induces the formation of antibodies capable to neutralize the lethal effects of the whole venom of *T.serrulatus*. We have isolated a full-lenght cDNA clone for a TsNTxP protein from a cDNA expression library using anti-TsNTxP antibodies. The nucleotide sequence of the gene that encodes TsNTxP was also obtained and revealed a presence of an intron whitin the sequence which codes for a signal peptide. Inicial attemps at expressing TsNTxP has been carried aout and the recombinant protein could be recognized by anti-TsNTxP antibodies.

O-28Tu

INVESTIGATIONS ON SEROTHERAPY AND IMMUNOPROTECTION AGAINST SCORPION TOXINS: RECOMBINANT TOXINS APPROACH

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InTunsia scorpion envenomation represents a real heath problem. Cases of death vary between 35 and 100 per year, mostly among children. *Androctonus australis hector* (Aah) and *Buthus occitanus tunetanus* (Bot) scorpions are frequently involved. To provide a full protection against these two species, three antigenic groups of toxins are to be neutralized by a therapeutic serum i.e. BotI, BotIII/AahII and AahI groups.

Three kinds of anatoxins preparations were used in our laboratory. One of these preparations is a recombinant α-toxin called BotXIVr. The antigen is a fusion protein with two synthetic domains (ZZ) of the protein A from *Staphylococcus aureus* efficiently expressed in *E.coli*. This fused protein is highly immunogenic in mice. Following immunization, raised antibodies recognizes weakly AahG50 fraction. Immunsera was tested for its capability to neutralize toxic fractions and toxins. It fully neutralizes 2DL50 of BotG50 fraction. Protection capacity induced in immunized mice was also tested; mice were fully protected whe challenged with 2DL50 of BotG50, in contrast AahG50 toxic fraction was toxic to mice.

BotXIVr displayed antigenic cross reactivity with BotI antigenic group.

Our investigations concerns actually two other antigenic groups i.e. BotIII/AahII and AahI groups.

O-29Tu

TREATMENT AND PREVENTION OF JELLYFISH ENVENOMATION

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Data on marine envenomation has been collected for ten years from tropical Australian Hospitals, Ambulance Stations and Surf Clubs. Recently, using E-mail, Internet and other direct contact, information on worldwide jellyfish distribution and sting occurrence has been obtained. Chirodropids occur worldwide in tropical and sub-tropical oceans. They cause many human deaths in the Indo-Pacific and have caused one in the western Atlantic; severe envenomation and morbidity is even more common. As their envenomation syndrome appears to cause similar, if not identical symptoms, first aid and medical treatments suggested should prove effective for all chirodropid envenomations, regardless of geographical location. Also, investigation into treatment of envenomation from the north Australian Irukandji (*Carukia barnesi*) should prove effective for similar syndromes that are discussed, causing severe human morbidity from other jellyfish species world-wide. Methods promoting awareness and prevention of jellyfish envenomation have contributed to the reduction of mortality from chirodropid envenomation in Australia by 30%, and even greater reductions of stings causing severe envenomation over the past ten years; such strategies should also prove effective in other geographic areas worldwide. Human deaths and severe morbidity from jellyfish envenomation emerge as a significant global medical problem. Despite this, research remains vestigial. The development of antivenom for Irukandji envenomation is current, but research into antivenom for other causes of mortality and morbidity after marine envenomation is essential. Undergraduate and post-graduate medical teaching remain conspicuous by their absence.

O-30Tu

ELISA FOR THE DETECTION OF TOXINS IN ACCIDENTAL ENVENOMING BY *ANDROCTONUS AUSTRALIS GARZONII*: CORRELATION WITH CLINICAL GRADATIONS.

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Scorpion stings are very frequent in Tunisia, yet a method for evaluating envenoming severity, and consequently victim treatment, has never been adequately established nor the efficiency properly evaluated. A performant sandwich ELISA was set up for measuring scorpion venom levels in the sera of accidentally envenomed humans in the aim to establish a quantitative relationship between these levels, envenoming severity and clinical symptoms. This assay was performed with polyclonal $F(ab')_2$ specific to *Androctonus australis garzonii*: (Aag) scorpion venom. It is simple, rapid, very sensitive (detection limit = 0.9 ng/ml) and is linear between 0.5 and 15 ng/ml of sera venom concentrations. The ELISA is also reproducible, with coefficients of variation determined at different venom concentrations (low: 4 ng/ml; medium: 8 ng/ml and high: 12 ng/ml) were lower than 10% in different sera collected from healthy donors.

An inquiry on scorpion sting envenomings was conducted from 1993 to 1996 in Tunisia to gather, accurate epidemiological, clinical and biological data from victims as well as treatment they received. Serum samples were collected from 308 well documented cases and were tested for their content of Aag venom by ELISA. They were classified in three grades of increasing severity defined according to clinical signs of envenoming:

- Grade I (GI: mild envenoming) identified by local signs only and absence of systemic symptoms.
- Grade II (GII: moderate envenoming) characterized by general signs without vital failures.
- Grade III (GIII: severe envenoming) concerned victims presenting, in addition of general signs, severe systemic symptoms with vital failures.

The quantification of scorpion venom antigens in sera of patients revealed a strong correlation between clinical symptoms and the level of venom antigens in serum. Indeed the mean sera venom concentrations were, respectively, equal to: 2.65 ± 0.81 ng/ml for G I, 9.79 ± 4.08 ng/ml for G II and 21.7 ± 6.51 ng/ml for G III envenoming.

O-31Tu

VOLTAGE CLAMP STUDIES OF *Bunodosoma granulifera* TOXIN ON THE OUTWARD MEMBRANE CURRENTS IN SNAIL NEURONS.

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Sea anemone toxins are considered valuable tools for characterizing the structure, function and the diversity of the voltage dependent Na^+ channels. Toxins affecting voltage dependent potassium channels have recently been detected in sea anemones (Aneiros et al., 1993, Castañeda et al., 1995, Schweitz et al., 1995). A variety of biologically active compounds have been found in the mucus secretion of *Bunodosoma granulifera* (Aneiros et al., 1993, Salinas et al., 1997). Among these, BgK is a peptide of 37 amino acids having a molecular weight of 4272. In this work the effects of BgK on outward K^+currents were studied in isolated neurons of the snail *Helix aspersa*, using whole cell patch clamp technique. To block Na^+ and Ca^{++} currents a external free Na^+ solution containing choline (70 mM) and 0.5 mM $CdCl_2$ was used. Application of BgK partially and reversibly blocked K^+ currents in the 1 pM to 1 µM concentration range (n=53). Dose-response curve for the current inhibition by BgK had the maximum effect at 1 nM (38 % at the peak and 59% at the steady state). Few experiments (n=8) were performed adding to the external solution 4-AP (1-10 mM) or TEA (100 µM). In presence of TEA and 4-AP, BgK (1 nM) action was almost unchanged, suggesting that BgK binds to another receptor site. Our results indicate that BgK is a potent, apparently non selective, K^+ channel blocker in molluscan neurons.

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Castaneda, O. et al. (1995) Toxicon, 33; 603-613.
Schweitz, H. et al. (1995) The Journal of Biological Chemistry, 270; 25121-25126.

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O-32Tu

ARE THE VOLTAGE-DEPENDENT SODIUM CHANNELS THE ONLY ONE TARGET FOR ALPHA SCORPION TOXINS?

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Voltage-dependent Na^+ channels are accepted unanimously as main targets for α scorpion toxins in insect as well as in vertebrate excitable tissues. These toxins modify the nerve conduction by slowing down Na^+ channel inactivation. Insect and mammal α toxins have been tested on cockroach isolated axon and neurosecretory dorsal unpaired median (DUM) neurones using current-, voltage- and patch- clamp techniques. A new type of channels, identified as background Na^+ channels (bNa) exhibiting a bell-shaped voltage dependence of open probability (Po), presents to α toxins a higher sensitivity than classic voltage-dependent Na^+ channels in the same preparation. The effect of α insect toxin (LqhαIT) is dose-dependent (threshold concentration of 10^{-8} M) and induces an increase of Po with a more pronounced bell-shaped voltage dependence and a transformation of single, brief, unclustered openings into bursts of very high current amplitudes. Detailed analysis of bursts indicates overlaping of several events, without modification of the control elementary conductance. At 10^{-7} M the bursts are condensated and separated by long silent periods. At 10 times higher concentrations, mammal α toxin (AaH2) has quite similar effects. The both α toxins induce high positive cooperativity between bNa channels. DUM neurone bNa modified α toxin transform a beating pacemaker activity into a pattern activity consisting of bursts of action potentials separated by slow prolonged predepolarizations. Such a transformation should alter the neurosecretory function of neurones.

O-33Tu

RELEASE OF ACETYLCHOLINE INDUCED BY TOXIN Ts-γ IS DUE TO STIMULATION OF Na^+ CHANNELS

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Previous data have shown that toxin Ts-γ , purified from the venom of the scorpion *Tityus serrulatus*, induces blockade of Na^+ channels (Barhanin *et al.*, 1984; Yatani *et al.*, 1988). These data do not explain the effects of toxin Ts-γ on the stimulation of cholinergic nerve fibers in isolated rat atria (Drumond *et al.*, 1995). In our present experiments, rat atria were placed in an organ bath containing Krebs solution before and after addition of 5 μM toxin Ts-γ , after labeling tissue ACh with 2 μCi/ml methyl[^{3}H]-choline chloride. The amounts of ^{3}H-ACh in solution after addition of toxin Ts-γ (N=7) were the following (DPM/mg tissue): 21.3 ± 3.5 (2 min), 41.5 ± 5.2 (5 min), 75.4 ± 6.9 (10 min) and 109.2 ± 5.3 (15 min), which were significantly different ($P< 0.01$) from the controls with no toxin added. Addition into the bath of 0.2 μM tetrodotoxin prevented the Ts-γ induced release of ACh, indicating that it depends on stimulation of Na^+ channels. However, in the frog sciatic nerve preparation mounted on a single sucrose-gap chamber, addition of 6 μM Ts-γ induced a progressive decrease in the amplitude of the compound action potential, achieving 50% reduction 30 min after addition of Ts-γ ,suggesting a decrease in the Na^+ conductance (N=3). To correlate the ACh release with the activation of Na^+ channels we performed whole-cell patch clamp experiments on GH3 cells maintaining the membrane potential at - 80 mV and depolarizing the cell to - 40 mV. During the perfusion of Ts-γ (0.3 μM) the Na^+ current increased by about 5 times as compared to control values (N=3). It is concluded that toxin Ts-γ favours stimulation of Na^+ channels at membrane potentials close to resting (physiological) levels and releases acetylcholine from vagal fibers in isolated rat atria.

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O-34Tu

ACTIVATION OF THE CALCIUM-DEPENDENT CHLORIDE CURRENT FROM *XENOPUS* OOCYTES BY MAITOTOXIN.

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Maitotoxin (MTX) is a polyether isolated from the dinoflagellate *Gambierdiscus toxicus*. MTX activates the influx of calcium in a wide variety of cells and stimulates phosphoinositide breakdown in some cell types. The mechanisms underlying the MTX effects are still unknown. In this study the effect of various concentrations of MTX (6-300 pM) were tested on the calcium-activated chloride current (I_{Cl}) from *Xenopus laevis* oocytes. MTX induced a concentration-dependent increase in the I_{Cl} with a half activation constant of 250 pM. This effect was completely reversible after perfusing the oocyte with MTX-free solution. Repeated applications of MTX followed by wash outs resulted in greater activation of I_{Cl}, suggesting that the MTX response is potentiated.

The results presented here show for the first time that: 1) MTX reversibly activates I_{Cl} in oocytes; 2) The MTX effect on I_{Cl} is potentiated by repeated applications of the toxin; 3) The oocyte system can be used to study the molecular mechanism leading to activation of calcium influx by MTX; 4) The oocyte system is a good model for the expression cloning of the MTX receptor obtained from cells with a higher toxin sensitivity.

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O-35Tu

GENOMIC ORGANIZATION OF SCORPION TOXINS ACTIVE ON K^+ CHANNELS AND EVIDENCE FOR A NEW CLASS OF TOXINS

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Toxins active on K^+ channels are minor components of scorpion venoms. They are single-chain polypeptides of 30 to 40 amino acid residues, with three disulfide bridges and share with toxins active on Na^+ channels (60-70 amino acid residues, four disulfides bridges), a common structural motif also found in the insect defensins: an α-helix linked to two anti-parallel β-sheets by two disulfide bridges. A 370 bp cDNA encoding the Kaliotoxin 2 (KTX_2) precursor (a 37 amino acid residues peptide purified from the North African scorpion *Androctonus australis* and acting as a high affinity blocker of K^+ channels), was previously obtained by PCR amplification. New cDNAs of scorpion toxins acting on K^+ channels was cloned from cDNA libraries made with venom glands of the North African scorpion *Androctonus australis* and the Brazilian scorpion *Tityus serrulatus*. The cDNAs of new long-chain peptides (64 amino acid residues containing six cysteins) were depicted in these two libraries. The sequences were homologous in their N-terminal part to a newly found scorpion toxin of *Tityus serrulatus* nammed AaH Kβ active on the voltage-sensitive channel K^+ "delayed-rectifyer". The sequences showed no homology with all either the long-chain or short-chain scorpion toxins already found. However, some homologies (45 %) raised between AaH Kβ, and a scorpion defensin purified from the hemolymh of the *Buthidae Leiurus quinquestriatus*. Thus, AaH Kβ was connected to the scorpion defensin phylum, using a multiple alignment with a CLUSTAL program.

At the genomic level, a single intron, close to the region encoding the C-terminal part of the signal peptide, was found in the gene of the short-chain scorpion toxins from *Androctonus australis* acting on various types of K^+ channels. The A+T content is particularly high (up to 86 %). The promoter sequences of the KTX_2 gene were studied. The transcription unit of the gene is 390 bp long. Thus, the genes of "short-chain" scorpion toxins active on K^+ channels are organized similarly to those of the long-chain scorpion toxins active on Na^+ channels and not like those of structurally related insect defensins, which are intronless.

O-36Tu

Possible mechanism/s of skeletal muscle degeneration by myoglobinuric-general myotoxins.

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The term myotoxin could be used to refer to local myonecrotic toxins (eg., taipoxin, notexin) or general myoglobinuric toxins (eg *Enh. Schistosa* VI:5, mulgotoxin). The mechanisms by which a general myotoxin acts systemically and affect many skeletal muscle group resulting in myoglobinuria is not fully understood. The possible mechanism/s of myoglobinuric mulgotoxin and PA myotoxin isolated from *Pseudechis australis* vehom were studied *in vivo* animal models. The earliest rise in creatine kinase after both local and systemic injection (i.p.) of mulgotoxin was noticed as early as 15 minutes. Electron microscopy revealed disrupted plasma membrane, shattering and degeneration of Z disc with myoglobin casts in the renal tubules. Immunohistochemistry showed loss of Z disc intermediate filament desmin by 15-60 min. Nuclear microscopic study showed early (1h) significant Ca and Na overload in the necrotic muscle fibers. This rise in Ca and myonecrosis was reduced by treatment with dantrolene but not by ouabain, an inhibitor of Na-K ATPase, showing that sarcoplasmic reticulum release of Ca^{2+} is involved in triggering this phenomenon. A rise in Na can activate Na modulated Ca release. Alkalinisation ($NaHCO_3$) of urine and dantrolene treatment showed protection from myonecrosis and myoglobinuria. We propose a working hypothesis that general myotoxin act through "calcium activated calcium overload" through "calcium mediated events" like activation of PLA_2 and neutral proteases, sarcolemmal injury and mitochondrial dysfunctions culminating in myoglobinuria (released from Z disc degeneration) and massive myonecrosis.

O-37Tu

DIFFERENTIAL ACTIONS OF PACIFIC CIGUATOXIN-1 ON VOLTAGE-GATED SODIUM CHANNELS OF MAMMALIAN SENSORY NEURONS: REVERSAL BY D-MANNITOL

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Ciguatera poisoning is characterised by short-term gastrointestinal disturbances and long-term sensory neurological symptoms such as paraesthesia and dysesthesia. This results from the ingestion of certain reef and semi-pelagic fish species which have bioaccumulated ciguatoxins through their marine food chain. Ciguatoxins (CTX) are a class of lipophilic polyether toxins, derived from the dinoflagellate *Gambierdiscus toxicus*, thought to cause repetitive firing of action potentials via a shift in the voltage-dependence of sodium channel activation. However there have been no studies of the effect of CTX on sodium channels in mammalian sensory neurons likely to be responsible for the sensory pathophysiology. Using whole-cell patch clamp recording of ionic currents in acutely dissociated rat dorsal root ganglion (DRG) neurons we have shown that CTX-1 isolated from the Pacific region (P-CTX-1) has differential effects upon the tetrodotoxin-sensitive (TTX-S) and tetrodotoxin-resistant (TTX-R) sodium channels in DRG neurons. P-CTX-1 produced a concentration-dependent reduction in peak amplitude of both TTX-S and TTX-R sodium currents and an increase in the leakage current in these cells. Additional actions of 5 nM P-CTX-1 on TTX-S sodium channels included a significant 12.8 ± 0.6 mV hyperpolarising shift in the voltage-dependence of activation and a 21.8 ± 4.1 mV hyperpolarising shift in steady-state sodium channel inactivation. However, in TTX-R sodium channels the main effect of 5 nM P-CTX-1 was a significant increase in the rate of recovery from sodium channel inactivation. These actions provide an explanation for the development of spontaneous action potentials thought to underlie the neurological symptoms associated with ciguatera poisoning. The effects of D-mannitol, the only clinically employed treatment for ciguatera fish poisoning, were also tested in this study. Perfusion with 50 mM D-mannitol completely reversed the hyperpolarising shift in steady-state inactivation seen in TTX-S sodium channels and reversed the increased rate of recovery from inactivation in TTX-R channels induced by P-CTX-1. This is the first study to demonstrate a direct effect of D-mannitol in reversing the action of P-CTX-1 on the gating mechanisms of mammalian voltage-gated sodium channels.

O-38Tu

EQUINATOXIN II INCREASES INTRACELLULAR CALCIUM AND CELL VOLUME IN NG108-15 CELLS AND IN SMOOTH MUSCLE CELLS

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Three cytolytic isotoxins have been isolated from *Actinia equina*: equinatoxin I, II, and III [1]. Low concentrations of equinatoxin II affect ionic currents of excitable membranes [2], and high concentrations cause cell lysis. As experimental animals die due to cardiorespiratory effects the aim of our study was to investigate the mechanisms of equinatoxin II induced vasoconstriction and cardiorespiratory arrest on the cellular level by monitoring intracellular calcium concentration and by analysing cell morphology. The time course of the change in intracellular Ca^{2+} concentration in smooth muscle cells and in hybrid neuroma-glioma (NG108-15) cells during the action of equinatoxin was measured using calcium sensitive fluorescent probe fura-2. Calcium-dependent alterations of cell morphology after application of equinatoxins were investigated by use of confocal laser scanning microscopy. Equinatoxin II (10 nM or higher) increased cytosolic Ca^{2+} concentration both in NG108-15 cells as well as in smooth muscle cells only in the presence of calcium in the bathing medium. Calcium channel blockers did not prevent the rise of intracellular Ca^{2+}. Similarly, the depletion of intracellular Ca^{2+} stores by thapsigargin had no effect on the action of equinatoxins. Simultaneously, cell morphology changed after application of equinatoxin II. Smooth muscle cells contracted, and the surface was covered with blebs. The size of NG108-15 cells increased during the action of the toxin. The swelling of the cells could be observed only in the presence of calcium in the extracellular fluid, and could not be prevented by treatment of the cells with tetrodotoxin. Results allow the conclusion that the rise of cytosolic Ca^{2+} is caused by calcium-permeable pore formation in cellular membranes, which consequently leads to the increase in intracellular volume.

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O-39Tu

COMPARISON AND CHARACTERIZATION OF THE VENOMS OF THREE *PARABUTHUS* SCORPION SPECIES OCCURRING IN SOUTHERN AFRICA

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Parabuthus transvaalicus, *P. granulatus*, and *P. villosus* are 3 medically important scorpion species occurring in southern Africa which can cause severe envenoming among people. In contrast to many other genera, no data is available on the venom composition of scorpions belonging to the genus *Parabuthus*. Here we have investigated the components which may contribute to the venomous potential. The constancy of venom composition within each of the 3 species and between the 3 species was investigated by means of gel filtration chromatography. The venoms of the 3 species each are characterized by a constant and typical elution pattern, resulting in a 'polypeptide fingerprint' which allows distinction between each species. Certain polypeptides in the venoms are common to either all 3 species, or to 2 of the 3 species. This points to a clear interspecies relationship within the genus. Several polypeptide toxins present in the venoms of *Parabuthus transvaalicus*, *P. granulatus*, and *P. villosus* were isolated and purified to homogeneity by means of ion-exchange and reversed phase chromatography. The purified toxins were characterized by electrophysiological screening using voltage and patch clamp on *Xenopus laevis* oocytes expressing voltage-activated (Kv1.1, HERG) and inward rectifier (Kir2.1) potassium channels, and on ventricular myocytes of the guinea-pig for studying voltage-activated sodium channels. Inhibitory polypeptide toxins uniquely acting on Kv1.1 channels, as well as polypeptide toxins which alter sodium channel gating have been identified in the 3 scorpion species.

O-40Th

Effects of Sorpion Venom on Central and Peripheral Circulatory Response in Opened-Chest Dog Model.

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Objective: Scorpion venom induced in dogs a rapid rise in systemic blood pressure (BP) and cardiac output (CO), followed by reduction of CO and BP within 1 hour. We tested the hypothesis that scorpion venom affects CO by reducing venous return to the heart.

Methods: Venous return was studied in 16 dogs, by measurements of CO, mean (venous) circulatory pressure and calculations of the pressure gradient and resistance to venous return, following injection of 0.05 mg/kg venom of the *Leiurus quinquestriatus* scorpion. In 8 of the 16 dogs, atropine (0.1 mg/kg) was given 15 minutes prior to venom injection (n=4) or 85 minutes (n=4) after venom administration.

Results: At 15 minutes, the venom induced an increase in BP (80%), and CO (250%) ($p<0.001$) with little effect on heart rate. At 90 minutes CO and heart rate declined considerably below baseline ($p<0.001$). Atropine prevented the decrease in heart rate, but had no effect on the reduction of CO. Five minutes after venom injection, mean circulatory pressure increased by 300% ($p<0.001$) accompanied by a rightward shift of the venous return curve with no effect on resistance to venous return. At 120 minutes mean curculatory pressure recovered and resistance to venous return remained 40 % ($p<0.01$) above baseline.

Conclusion: This study indicates that in dogs scorpion venom affects cardiac output by modifying the deteminants of venous return. The initial increase in cardiac output is related to increased mean circulatory pressure since resistance to venous return did not change. The later fall in cardiac output is related to the reduction of mean circulatory pressure and increased resistance to venous return.

O-41Th

Cardiovascular Effects In Dogs Following Injection of Venom From the *Leiurus quinquestriatus* Scorpion.

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Objective: The present study tested the hypothesis that scorpion sting induces left ventricular (LV) hypokinesia and myocardial ischemia shortly after venom injection due to reduction of coronary blood flow and increased oxygen demand.

Methods: In 5 mechanically ventilated, open-chest dogs, we measured LV function following IV injection of venom (0.05 mg/kg) obtained from the *Leiurus quinquestriatus* scorpion. Hemodynamic responses to the venom were followed up for 90 minutes.

Results: The venom induced significant combined respiratory and metabolic acidosis (arterial pH progressed from 7.35 ± 0.03 at baseline to 7.10 ± 0.06 at 90 minutes). There were large increases in blood pressure, LV end systolic pressure, stroke work, and velocity of contraction. Twenty minutes following venom injection, cardiac output increased by 37% but then declined to 36% below baseline by 90 minutes ($p < .05$). Coronary blood flow increased significantly in proportion to increased perfusion pressure; hence, there was no change in coronary vascular resistance. There was no evidence of myocardial ischemia or LV dysfunction since there was no change in myocardial pH, percentage fiber shortening, or LV end-diastolic pressure. Despite the fact that some variables returned to baseline at 90 minutes, they did not reach steady state; thus, the preparation would have continued to deteriorate.

Conclusion: Myocardial ischemia does not occur in this dog model following administration of scorpion venom. Initial increased and late decreased candiac output is probably secondary to peripheral circulatory effects of the venom.

O-42Th

Scorpion venom leads to gastointestinal ischemia in pigs despite increased oxygen delivery.

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Objective: Scorpion envenomation may cause metabolic acidosis. We tested the hypothesis that acidosis is related to ischemia of the gastrointestinal tract.

Methods: 10 intubated, sedated and instrumented pigs, were given IV 0.05 mg/kg *Leiurus quinquestriatus* venom. Measurements were made before and 5, 15, 30, 60, 120, 180 and 240 min. following venom injection.

Results: Within 5 min. there was an increase in blood pressure (BP) (97 ± 18 to 146 ±47 torr, p<.001), heart rate (HR) (70 ± 12 to 121 ± 24 bpm, p<.001), and cardiac output (CO) (1.8 ± 0.35 to 2.95 ± 0.53 L/min, p<.001). There were significant decreases in systemic and pulmonary vascular resistance. Arterial and gastric mucosal pH decreased - 7.40 ± 0.04 to 7.25 ± 0.07, p<.0001; for arterial and 7.33 ± 0.08 to 7.17 ± 0.13, p<.00001, for gastric pH - by 30 min. The decrease in arterial pH was not sufficient to account for the change in gastric mucosal pH, indicating gastric mucosal ischemia. Arterial lactate increased from 2.6 ± 1.4 to 7.4 ± 1.9 (p<.001). There were large increases in serum epinephrine and norepinephrine values by 5 min. All hemodynamic variables and catecholamine levels returned to baseline by 4 hours. Oxygen delivery remained normal or supernormal for 4 hours following envenomation. However, in spite of this, systemic and gastric pH changes indicate impaired gastrointestinal O_2 delivery.

Conclusion: In spite of increased peripheral O_2 delivery, scorpion envenomation induced ischemia of the gastrointestinal tract associated with metabolic acidosis. This may be explained by redistribution of blood flow due to massive release of catecholamines.

O-43Th

LACK OF EFFECT OF *Tityus discrepans* SCORPION VENOM ON ISOLATED RABBIT LUNG

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Tityus discrepans scorpion produces a lung edema in 32% of the human victims and this causes 25% of the casualties. We studied the mechanism of production of a lung edema by *T. discrepans* venom in rabbits (*Oyctolagus cuniculus*, ♂, 2 Kg) *in vivo* and in rabbit's isolated lungs. Isolated lungs were dissected out and perfused with 250 ml BSA-Krebs saline, (HEPES buffered pH 7.4). One mg/kg_{rabbit} scorpion venom was added to this solution after assuring that the preparation was in good condition. No significant differences in fluid filtration rate, or the pressures in pulmonary artery, left atrium or trachea were found between experimental and control rabbits. Light microscopy showed that the venom produced no changes in isolated lung structure. On the other hand, light microscopy of rabbit lung sections after injecting the animals i.p. with 1 mg/kg of scorpion venom showed a severe edema with intra alveolar hyaline membranes, abundant micro thrombi in lung vessels, abundant intra bronchial, intravascular and intra alveolar lymphocytosis and thickening of inter alveolar septa. Rabbits that received 4.000 units of heparin 30 minute before the i.p.venom injection, and then twice every 12 h, did not suffer a lung edema when injected with venom. Animals receiving 15 mg/kg ajoene i.p. 30 minutes prior to venom injection and then twice every 12 h, were not protected against venom-induced lung edema. These results support the notion that *T. discrepans* venom produces a lung edema through an indirect mechanism which probably encompasses the blood coagulation cascade but does not involve platelet activation.
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O-44Th

NEUTRALIZATION OF THE MYOTOXIC AND NEUROTOXIC ACTIVITIES OF *Crotalus durissus terrificus* VENOM AND OF ITS PRINCIPAL COMPONENT, CROTOXIN

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Crotoxin (CrTX), the principal component of South American rattlesnake (*Crotalus durissus terrificus*, Cdt) venom, possesses both myotoxic and neurotoxic activities. In this study, we have investigated the ability of commercial anti-crotalic antivenom (CA, produced in horses) and specific antivenoms (anti-Cdt and anti-CrTX, raised in rabbits) to neutralize the physiological, biochemical and morphological changes induced by Cdt venom and CrTX in the phrenic nerve-diaphragm (PND) and *extensor digitorum longus* muscle (EDL) preparations of mice. Muscle contractions were induced with supramaximal stimuli (0.2 ms; 0.1 Hz).. In some cases, the venom/toxin (10 µg/ml) to be added was pre-incubated with the desired antivenom for 30 min at 37°C prior to testing. In control experiments, Tyrode solution alone was incubated with the antivenom. At 0, 15, 30, 60, 90 and 120 min after the above additions, 100 µl aliquots of the bathing solution were withdrawn from the organ bath and the levels of creatine kinase (CK) determined. At the end of 120 min, the preparations were washed and fixed in Bouin solution prior to processing for histological analysis. The time (in min) required to produce 50% neuromuscular block in the PND and EDL preparations was, respectively, 103±9 and 59±6 for Cdt venom and 75± 9 and 110±7 for CrTX. The antivenoms dose-dependently inhibited this neuromuscular activity of the venom and CrTX. At the end of a 120 min incubation, the CK levels for Cdt venom and CrTX were, respectively, 290 IU/l and 1020 IU/l. All of the antivenoms effectively neutralized the ability of CrTX, but not of Cdt venom, to release CK. Based on light microscopy analysis, the anti-CrTX antivenom protected the preparations against the myotoxic action of both Cdt venom and CrTX. The predominance of fast fibers in the EDL and slow fibers in the PND preparations appears to be the principal cause of the differences observed between the effects of Cdt venom and CrTX. The ability of anti-CrTX antiserum to neutralize both the myonecrosis and the neuromuscular block caused by this toxin suggests that the antibodies recognize the site(s) responsible for these activities.

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O-45Th

PHYSIOLOGICAL BASIS OF NEUROMUSCULAR PARALYSIS CAUSED BY TOXIC PHOSPHOLIPASES A_2.

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Victims of bites by taipans, kraits and some other elapid snakes exhibit a severe and long-lasting paralysis (Pearn, 1971; Connolly et al. 1995; Warrell et al. 1983, for example). The clinical features of paralysis are rapid onset, a period of stable but profound weakness and a third phase of slow recovery of function. The toxins responsible for this weakness are generally considered to be the presynaptically neurotoxic phospholipases. These toxins are usually defined as toxins that inhibit transmitter release to initiate neuromuscular failure.

Mammalian neuromuscular preparations exposed to β-bungarotoxin exhibited an initial enhancement of release, that was associated with a clustering of transmitter vesicles around the periphery of the motor nerve terminal. Neuromuscular failure followed, and was associated with the depletion of transmitter vesicles from the nerve terminal. The concept that the toxins inhibit transmitter release is clearly wrong.

Studies in vivo confirmed the findings on isolated neuromuscular preparations, but also demonstrated the extensive damage caused by the β-bungarotoxin to the motor nerve terminal and the motor axons, with clear degradation of the axonal cytoskeleton. There was no damage caused to the skeletal muscle. Axonal regeneration began at 3 days and was complete - though still exhibiting abnormal morphology - at 21-28 days.

The data suggest that the rapid onset of weakness is due to the depletion of transmitter from motor nerve terminals. The period of profound weakness is due to the destruction of motor nerve terminals and motor axons and the period of slow recovery is due to the regeneration of the motor axons and neuromuscular junctions.

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O-46Th

REORGANISATION OF MOTOR INNERVATION OF SKELETAL MUSCLE EXPOSED IN VIVO TO β-BUNGAROTOXIN

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Long term neurotoxicity is a prominent clinical feature following envenomation by kraits (Pearn, 1971; Warrell et al. 1983; Pawar and Singh, 1987; Theakston et al. 1990, for example). The 'typical' patient exhibits profound weakness for up to four or five days followed by a slow recovery that may last a further two or three weeks.

The principle toxins involved in neurotoxicity are the β-bungarotoxins. These toxins cause the depletion of transmitter, damage the motor nerve terminal and damage the intramuscular axons (Abe et al. 1976; Dixon, 1996).

The slow recovery from paralysis probably reflects the regeneration of the motor axons. This was studied using a combination of immunocytochemical studies combining the imaging of junction ACh receptors and axonal neurofilaments, and histochemistry to determine the distribution of metabolically distinct muscle fibres in a mixed muscle.

Soleus (SOL) and extensor digitorum longus (EDL) muscles were exposed to a single injection of β-bungarotoxin (10 μg). Between 5 days and 7 months later, the muscles were removed and used for histochemistry (ATPase at pH 4.3) or immunocytochemistry.

At early stages, axon sprouts, emerging from nerve terminals and from nodes, were common. Many of these sprouts made synaptic contact with adjacent muscle fibres leading to a high incidence of collateral sprouting. Histochemical staining also indicated a higher than normal degree of fibre type grouping, consistent with extensive collateral sprouting. These abnormal patterns of innervation lasted for at least 7 months.

It is concluded that the re-construction of neuromuscular junctions following exposure to the presynaptically active neurotoxic phospholipases is not a simple restitution of the original *status quo*.

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O-47Th

PALYTOXIN AND TRANSMITTER RELEASE IN THE SKELETAL NEUROMUSCULAR SYSTEM

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Palytoxin is the most potent natural toxin so far isolated. It is a complex polyalcohol with 5 sugar moieties and amino and hydroxy terminals. It has been suggested that palytoxin binds to Na^+K^+ ATPase, inhibiting its activity and converting it into an ionophore (Habermann, 1989; Hirsch and Wu, 1997).

We studied the effects of palytoxin on transmitter release in the murine neuromuscular system. The toxin was inactive at concentrations $<10^{-8}$M. At concentrations of $3x10^{-8}$M to 10^{-7}M it first enhanced transmitter release and then blocked transmission completely. Action potential conduction in the axons was unaffected, ACh receptors were not blocked and ACh synthesis was unimpaired. Electron microscopy revealed nerve terminals that were swollen and devoid of transmitter vesicles except for some clathrin coated vesicles typical of recycled vesicles in excessively stimulated preparations.

Palytoxin did not inhibit neuronal Na^+K^+ ATPase activities at pharmacologically active concentrations. Even at 10^{-5}M Palytoxin only inhibited ATPase activity by about 10%. Ouabain binds to Na^+K^+ ATPase; palytoxin inhibited the specific binding of ^{3}H-ouabin, but this inhibition only occurred at concentrations of palytoxin $>10^{-7}$M (i.c.$_{50}$ $4x10^{-6}$M).

The Na^+K^+ ATPase may be involved in the binding of palytoxin, but in neuronal tissue its role in the depletion of transmitter is minimal.

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O-48Th

BLOOD PLATELET ACTIVATION BY VENOM OF AFRICAN GREEN BUSH VIPER *(ATHERIS SQUAMIGER)*

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The venoms of African bush vipers *(Atheris)* are poorly studied, however, one fatal case in Central African Republic and recently a serious envenomation by *Atheris squamiger* kept in Germany were reported. In both cases a severe haemorrhagic diathesis was the main clinical problem. LD_{50} of our sample of the *A. squamiger* venom was 5 mg/kg (mice, s.c.). The venom produced local haemorrhage corresponding to about 25% of the activity of puff adder venom *(Bitis arietans)*. *In vitro* the venom had no direct, but factor V and X dependent prothrombin-converting activity. It also exhibited a strong fibrinogen-converting and platelet-aggregating activity. The later activity was not a protease and was also effective on aspirin-treated platelets. Rat serum rapidly inhibited the platelet-aggregating activity of the venom, human serum, however, had only a slow partial inhibitory effect. The preliminary experiments showed, that platelet-aggregating activity may be separated from fibrinogen-converting activity by anion-exchange (Mono Q) FPLC-chromatography. In experiments with venoms of four *Atheris* species the *A. nitschei* venom exhibited about 100times higher, *A. superciliaris* approximately the same, and *A. chlorechis* only very low platelet-aggregating activity, as compared to *A. squamiger* venom. Supported by the grant 3051-3 of IGA MH Czech Republic.

O-49Th

HOW DOES HONEY BEE VENOM KILL?

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Honey bee venom is the best characterized insect venom and possesses several toxins including the peptide melittin, the enzyme phospholipase A_2 (PLA_2), and the peptide apamin. Nevertheless, the main toxin responsible for causing death in mammals is uncertain, as is the mechanism of death from toxic envenomation. No antivenin for bee venom has been developed and the crucial toxin to be neutralized is yet to be positively identified. Hyperimmune beekeeper serum has little ability to neutralize bee venom lethality (*Am. J. Trop. Med. Hyg.* 43, 79-86 [1990]) despite containing elevated levels of anti-PLA_2. Among other effects, PLA_2 is known to increase vascular permeability and cause pulmonary congestion. Melittin, often described as a hemolysin, is a true cardiotoxin. Based on i.v. venom and toxin challenge experiments, I suggested (*Toxicon* 33, 917-27 [1995]) that melittin via its cardiotoxicity was the cause of death in envenomed animals. However, pathological examination of dogs lethally attacked by honey bees and one human patient revealed moderate to severe pulmonary congestion with little observable effect on the heart or other organs. A dual approach using i.v. venom and natural stings in mice was chosen to continue analysis of the cause of death by bee venom. Lethality plus appearance and weight of the lungs in mice that died were measured. The results from both challenge routes revealed a mixed picture. In some animals the lungs were red, congested and heavy, in others they were white, light, and with little congestion. These results suggest that intravenous challenge does not necessarily kill via a bolus of toxin blocking heart function, and can provide useful information to model the natural intoxicating process. The cause of death may be either direct cardiotoxicity, or pulmonary congestion subsequent to cardiac failure and the exact cause in individuals might depend upon the physiology of the particular challenged individual. Finally, to develop an effective antivenin, melittin must be neutralized.

O-50Th

ISOLATION AND PARTIAL AMINO ACID SEQUENCE OF A NOVEL METALLOPROTEASE FROM THE VENOM OF THE COLUBRID SNAKE *HYDRODYNASTES GIGAS* (FALSE WATER COBRA)

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Perhaps half of the species in the huge and polyphyletic snake family Colubridae produce specialized secretions (venom) from a Duvernoy's gland, but very little is known about their venoms. The Colubridae contains many lineages of mildly to highly venomous snakes which are not related to the front-fanged venomous snakes, and colubrid venoms are therefore likely sources of novel biological compounds. A recent method to increase yields of venoms from rear-fanged colubrids (Hill and Mackessy, *Toxicon* 35(5):507) was utilized on *Hydrodynastes gigas*. Bites from this large (up to 2 m) species have been observed to produce severe edema, tissue necrosis and pain, and a published LD_{50} of 2.0 mg/kg suggested a toxicity in the range of many rattlesnake venoms. Venom obtained from *H. gigas* showed high caseinolytic protease activity and catalyzed the degradation of fibrinogen, but it did not show significant PLA_2, phosphodiesterase, hyaluronidase, L-amino acid oxidase, thrombin-like or kallikrein-like activities. Lyophilized venom was subjected to gel filtration (Sephadex G-200), reversed phase HPLC and electrophoretic purification procedures, yielding a 29 kD caseinolytic protease. The enzyme was inhibited by EDTA and 1,10-phenanthroline, indicating that it is a metalloprotease, and it showed extremely high levels of activity when analyzed on gelatin-SDS PAGE. It produced moderate levels of hemorrhage and myonecrosis when injected in mice. Automated N-terminal protein sequence analysis identified the first 20 residues and showed that the enzyme is not N-terminally blocked (as are many crotalid venom metalloproteases). The enzyme showed no sequence homology with known elapid, viperid or crotalid venom proteases, and since *H. gigas* is only distantly related to the front-fanged venomous snakes, this protease may represent a novel subclass of venom metalloproteases. Identification of the metal cofactor, substrate specificity and further sequencing studies are currently in progress. This work was supported by grant #1-R15-GM52665-01 from the NIGMS section of the National Institutes of Health.

O-51Th

A NEW SK_{Ca} CHANNEL TOXIN FROM *OXYURANUS SCUTELLATUS* TAIPAN VENOM

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Taicatoxin (M_r 52,000), isolated from the venom of the Australian taipan snake *Oxyuranus scutellatus* is an oligomeric complex of three non-covalently linked polypeptides (α-neurotoxin, phospholipase A_2 and protease inhibitor). Taicatoxin has previously been regarded as a specific blocker of high threshold calcium channels in heart. We have discovered that taicatoxin also blocks apamin-sensitive, small conductance Ca^{2+}-activated potassium channels (SK_{Ca} channels) in both chromaffin cells and in the brain. Other calcium channel blockers (e.g. ω-conotoxins, agatoxin IVA, calciseptine and many dihydro-pyridines) have no effect on SK_{Ca} channels. Taicatoxin recognises ^{125}I apamin acceptor sites on rat brain synaptosomal membranes with high affinity (K_i = 1.45±0.22nM) and also specifically blocks affinity labelling of a 33kDa ^{125}I-apamin binding polypeptide on rat brain membranes (putative SK_{Ca} channel β-subunit). Taicatoxin (50nM) completely blocks the apamin-sensitive after-hyperpolarising slow potassium tail currents in rat chromaffin cells (mean block 97±3%, n=12), while only partially reducing total voltage-dependent calcium currents (mean block 12±4%, n=6). Taicatoxin has no effect on delayed rectifier currents, fast tail currents and other outward currents in chromaffin cells, suggesting that it does not block other apamin-insensitive potassium channel subtypes, including charybdotoxin-sensitive, high conductance calcium-activated potassium channels. It is thought that a dominant contribution to the pharmacophore in apamin, neuromuscular blockers and scorpion toxins (e.g. scyllatoxin) that is responsible for SK_{Ca} channel block, consists of two positive charges, 11Å apart (provided by two adjacent Arg residues in the peptide toxins). Taicatoxin contains an Arg-Arg motif consistent with the recognition of SK_{Ca} channels. In view of these findings, the use of taicatoxin as a specific ligand for calcium channels should now be reconsidered.

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O-52Th

Epitope mapping of the nicotinic acetylcholine receptor using photoaffinity derivatives of α-neurotoxins from Cobra venom

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α-Neurotoxins from Cobra and Krait bind with very high affinity and specificity to peripheral and some neuronal nicotinic acetylcholine receptors (nAChR). We produced arylazido derivatives which carry the photoactivatable group in specified positions of the peptide toxin. With one such derivative we showed that the competitive antagonist-binding site is less than 15 Å away from the ion channel (1), that the binding site spans the receptor from the outside to the lumen, forming an intersubunit gorge (2), and that the subunit arrangement is (clockwise) $\alpha_H\alpha_L\beta\gamma\delta$ (3). We conclude that photoactivatable toxin derivatives are powerful tools for elucidating structural features of the receptor. One difficulty is the low yield of the photocross-links obtained which poses the problem that for the identification of the site of the cross-link on the receptor one has to separate a large (about 20fold) excess of unlabeled receptor protein from small amounts of cross-linked protein. To overcome this difficulty the toxin (α-neurotoxin II from *Naja naja oxiana*) was first derivatized with the NHS-azidobenzoic acid ester. The derivative carrying the label in position K26 was chosen to be biotinylated. After photocross-linking this 'double derivative' with the receptor (nAChR from *Torpedo californica* electric tissue) the subunits were separated by preparative SDS-PAGE and the cross-linked subunits were isolated with a streptavidin column. For the elution from the column we used TCEP (Tris [2-carboxyethyl-phosphine as the reducing agent.

1.) Machold et al. *Proc.Natl.Acad.Sci. USA* 92, 7282-7286, 1995
2.) Hucho et al. *Eur.J.Biochem* 239, 539-557 (1996)
3.) Machold et al. *Eur.J.Biochem.* 234. 427-430. 1995)

O-53Th

Specific neurotoxins interaction on muscular and neuronal (α7) nicotinic acetylcholine receptor

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Snake curaremimetic toxins are currently classified as short-chain and long-chain toxins according to their size and the number of disulfide bonds, 60-62 residues and 4 disulfide bonds in one hand and 66-74 and 5 disulfide bonds on the other hand. All the toxins bind with high affinity (10^{-10}-10^{-11} M) to the peripheral nicotinic acetylcholine receptor (AChoR) at the exception of neuronal toxins which interact specifically with the neuronal receptors (especially the α3β2 type). Our studies have demonstrated that long-chain and short-chain toxins present in fact major biological differences. Thus, by binding experiments on HEK cells expressing a chimaeric construction of the neuronal α7 receptor, we pointed out an affinity that is nearly four orders of magnitude higher for long-chain toxins as compared to the short-chain toxins. Electrophysiological experiments done with the wild-type and chimaeric α7 receptors confirmed these results. Moreover, we demonstrated that this discriminating property is associated with a specific differences between long-chain and short-chain toxins, i.e. the additional cyclized loop at the tip of their central loop. Our findings suggest that functional classification of toxins that bind to AChoRs should probably be done regarding their activities on both peripheral and neuronal receptors.

O-54Th

NATURAL LIGANDS WITH SELECTIVITY FOR SUBTYPES OF MUSCARINIC RECEPTORS

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More than 12 different proteins that bind to muscarinic receptors (MAChR) have been isolated from *Dendroaspis* snake venom. They were named muscarinic toxins (MTs). MT1 and 2 (1, 2) from *D. angusticeps* venom bind selectively to m1 (K_i= 50-400nM) and m4 (K_i=130-1200nM) cloned human MAChR (3), while MT3 (4), or m4-toxin (5), exhibits the highest affinity for m4 receptors (K_i=0.95nM). MT1 and 2 were also tested in native membranes from rat. High affinity binding was found in the hippocampus (rich in m1 and m4 subtypes) and in the striatum (rich in m4), but no interaction was found with pancreas membranes (rich in m3) nor with atria (rich in m2). Unexpectedly, MT1 and 2 were able to displace ^{3}H-prazosin, an α-adrenoceptor antagonist (K_i=0.3μM) in cerebral cortex. However, this binding was reversible, while the binding to MAChR appears irreversible. The toxins have been labelled by using ^{3}H-acetic anhydride. The autoradiograms of rat brain slices showed a specific binding pattern for each toxin. In pharmacological experiments carried out in rabbit vas deferens, MT1 and 2 behaved as M_1 muscarinic agonists, while MT3 had no effect on responses to neural stimulation. The muscarinic toxins, with their high selectivity, high potency and slow reversibility or irreversibility, seem to be exceptionally useful tools for studying the functional roles of individual muscarinic receptor subtypes.

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2. Jerusalinsky *et al.* (1989) J. Neurochem. 59: S92.
3. Jerusalinsky and Harvey (1994) Trends Pharmacol. Sci. 15: 424-430.
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O-55Th

DECREASED COLLAGEN-STIMULATED PHOSPHORYLATION OF THE PLATELET TYROSINE KINASE pp72^{syk} BY THE VENOM METALLOPROTEINASE JARARHAGIN

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Early signaling events in platelet stimulation by collagen leading to secretion and aggregation include the phosphorylation of both tyrosine kinase pp72^{syk} and phospholipase C gamma 2, followed by activation of protein kinase C (PKC) and serine/threonine phosphorylation of pleckstrin (p47). Platelet aggregation can be inhibited by the RGD-containing snake venom disintegrins through the high-affinity binding to the platelet $\alpha_{IIb}\beta_3$ integrin. Venom haemorrhagic metalloproteinases can also inhibit platelet aggregation but the mechanism is not entirely clear. We recently found that jararhagin, a 52-kDa metalloproteinase, selectively inhibits collagen-induced platelet secretion and aggregation, by recognizing the I domain of α_2 subunit and cleaving β_1 subunit of the platelet collagen receptor, $\alpha_2\beta_1$ integrin. This study demonstrates that the cleavage results in the abolition of collagen-induced phosphorylation of the protein tyrosine kinase pp72^{syk}; such inhibition is not observed in the presence of two RGD-containing disintegrins (contortrostatin and echistatin). In addition, stimulation by ADP can restore collagen-induced platelet aggregation of jararhagin-treated platelets only. This demonstrates the interaction of collagen with an intact ADP-activated $\alpha_{IIb}\beta_3$ integrin in jararhagin-treated platelets which also restores phosphorylation of pp72^{syk} and pleckstrin. We conclude that disintegrins and jararhagin inhibit collagen-induced platelet aggregation by different mechanisms. Disintegrins do not interfere with stimulation signaling but inhibit aggregation through occupancy of $\alpha_{IIb}\beta_3$ integrin. Jararhagin affects signaling, probably by degrading the β_1 subunit of platelet $\alpha_2\beta_1$ integrin, without interfering with $\alpha_{IIb}\beta_3$ integrin.

O-56Th

SRTX-d/e INHIBITS SARAFOTOXIN-b-INDUCED CONTRACTION OF GUINEA PIG INTESTINE

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The sarafotoxins (SRTXs), isolated from the venom of *Atractaspis*, and the endothelins (ETs), regulatory peptides functioning in mammals and other vertebrate, show a high degree of sequence homology. However, even small structural differences in the various isopeptides of this ET/SRTX family are manifested in pharmacological activity. For instance, SRTX-d/e, which is essentially non-toxic, has three substituted residues, as compared to the most toxic peptide of *A. engaddensis*, SRTX-b. SRTX-d/e was found to be a very poor vasoconstrictor, yet its binding affinity to rabbit aorta preparation was similar to that of SRTX-b. SRTX-d/e is also a very poor contractile agent in the guinea pig ileum. Concentrations below 15 nM at 37°C did not induce any contraction when tested on isolated ileal segments; at 500 nM the induced contraction was 40% of the effect induced by 15 nM SRTX-b. Preincubation of guinea pig ileal segments with SRTX-d/e showed a dose dependent inhibition of SRTX-b-induced contraction. It has been shown that SRTX-b and ET-1 induce similar contraction of guinea pig ileum. However, whereas, contraction induced by 15 nM SRTX-b was completely abolished by 25 nM SRTX-d/e, complete inhibition of contraction of the ileal segments induced by 15 nM ET-1 was achieved only after preincubation with 500 nM SRTX-d/e. Competition binding experiments with ileal membranes revealed no differences in the displacement of ^{125}I-labeled SRTX-b or ET-1 by SRTX-b, ET-1 or SRTX-d/e. It is thus suggested that the different potencies of SRTX-d/e as antagonists of ileal smooth muscle contraction by SRTX-b or ET-1 are due to two different receptor subtypes; one of which is probably coupled more efficiently to the smooth muscle contraction cascade upon binding to ET-1.

O-57Th

A PEPTIDE LIGAND FOR GLUTAMATE RECEPTORS FROM THE VENOM OF THE TARANTULA Phlogiellus sp.

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A variety of peptide toxins have been isolated from spider venoms, some of the most interesting have been found in tarantula venoms. Tarantula peptide toxins mostly target voltage-dependent sodium, calcium or potassium channels.
Toxicity screening of 35 tarantula venoms by intracerebroventricular injection in mice has demonstrated unusual toxicity symptoms for the venom of *Phlogiellus sp.*, a tarantula from Papua New Guinea. Injected mice became lethargic and died rapidly without any of the excitotoxicity symptoms commonly observed for other venoms. The major peptide component of the venom (Phlotoxin1 - PhlTX1) was purified by HPLC and showed activity similar to the crude venom at picomolar doses. PhlTX1 is a single-chain peptide (MW 4058.65) consisting of 34 amino-acids linked by 3 S-S bonds. It has 48% sequence homology with huwentoxin I, isolated from the Chinese tarantula *Selenocosmia huwena*.
According to intoxication symptoms, activity against post-synaptic receptors was assumed. PhlTX1 was able to bind to NMDA, AMPA and kainate-type glutamate receptors (GluR) from rat brain with an affinity comparable to that of Glu.
Although its affinity is lower than some synthetic agonists/antagonists, PhlTX1 is the first peptide ligand isolated from a natural source binding to a non-polyamine-sensitive GluR site. It may be a useful tool for the study of the glutamate receptors.

O-58Th

INTERACTION OF TX1, A NEUROTOXIN FROM THE SPIDER *Phoneutria nigriventer* VENOM, WITH THE GUINEA PIG ILEUM

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The neurotoxin Tx1 isolated from the venom of the South American spider *Phoneutria nigriventer* produces tail elevation, excitation and spastic paralysis of posterior limbs after intracerebralventricular injection in mice. As Tx1 produces ileum contraction in bioassay (Cordeiro et al *J. Toxin Rev* 14(3): 309, 1995), we have investigated its effect on the acetylcholine release and the binding of the radioiodinated toxin on the preparation of myenteric plexus-longitudinal muscle membrane (MPLM) from guinea pig ileum. ^{125}I -Tx1 binds specifically and reversibly to a single class of noninteracting binding sites (n_H =1.1) of high affinity (Kd= 3.5 x 10^{-10} M) and low capacity (1.2 pmol/mg protein). In competition experiments with several drugs (including ligands of ionic channels) only PhTx2 and PhTx3 competed with specific binding site of ^{125}I-Tx1 ($K_{0.5}$= 9.56 x 10^{-9} and 1.58 x 10^{-7} M, respectively). PhTx2 and PhTx3 are fractions from the same spider venom that contain toxins acting on sodium and calcium channels (Araujo et al *J. Physiol.* 467,1993 and Romano-Silva et al *Anais SBBq*, 1995), respectively. On the other hand, the neurotoxin PhTx2-6, one of the isoforms of the PhTx2 pool did not affect the ^{125}I-Tx1 binding. Tx1 reduced 33% the ^{3}H-Ach release evoked by PhTx2 pool, but it did not affect the basal ^{3}H-Ach release neither that induced by veratridine or KCl. Considering our results, the homology of Tx1 with toxins acting on calcium channels (ie, ω -Aga IA and IB, Diniz et al *J Biol. Chem.* 268(21): 15340, 1993) and its competition with ^{125}I-ω-Cono GVIA in the central nervous system (De Lima et al, personal communication), we suggest that Tx1 target site can be related to the calcium channels.

O-59Th

A NOVEL FOUR DISULFIDE-BRIDGED SCORPION TOXIN, WITH HIGH AFFINITY TOWARDS VOLTAGE-GATED K⁺ CHANNELS.

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We isolated a new peptidic toxin from the venom of the Scorpionidae *Heterometrus spinnifer*, and named it HsTX1. It is constituted by a chain of 34 amino acids, reticulated by 4 disulfide bridges. Its sequence is 32 to 47% identical to that of classical 3 disulfide-bridged scorpion toxins acting on voltage-gated potassium channels, whereas it is 53 and 59% identical to that of the 4 disulfide-bridged toxins, Pi1 and maurotoxin, recently isolated from the venom of two other Scorpionidae. The carboxylic and amidated C-terminal forms of HsTX1 were synthesized, and the four disulfide bonds readily oxidized. Identity between the amidated synthetic peptide and the natural one was proved by co-elution from C18-HPLC, MALDI-TOF mass spectrometry in reflector mode and blocking activity on the rat Kv1.3 channels expressed in *Xenopus* oocytes. The disulfide bridges of synthetic and natural toxin were studied by limited reduction-alkylation at acidic pH and sequencing. The two extra half-cystines in HsTX1, as compared to classical 3 disulfide-bridged scorpion toxins, are cross-linked. We assume that the three other disulfide bonds are homologous to the ones that reticulate classical K^+ channel scorpion toxins. Molecular modeling of HsTX1, using the parameters obtained for ChTX, shows that it adopts the same overall fold as other scorpion K^+ channel blockers, the fourth disulfide introducing a further stabilization. HsTX1 blocks reversibly the rat Kv1.3 channels, with an IC_{50} of about 12 pM. It also competes with ^{125}I-Kaliotoxin for binding to the voltage-gated K^+ channels in rat brain synaptosomal membranes with an IC_{50} of ~ 1 pM, whereas it does not compete with ^{125}I-Apamin for its binding on the same preparation. We conclude that HsTX1 is one of the most active voltage-gated K^+ channel blockers, and the third member of a new structural type of scorpion toxins.

O-60Th

FIBRIN GLUE DERIVED FROM SNAKE VENOM: USE IN DERMATOLOGICAL SURGERY

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The search for an ideal adhesive to replace suturing has been pursued for years. Several papers in literature reported on application of collagen gel, cyanoacrilates and conventional fibrin glue. All of these substances present undesirable effects which prevent their use in medical practice. In 1989, researchers from the ***Center for Study of Venoms and Venomous Animals of São Paulo State University - CEVAP-UNESP***, developed a new adhesive composed of animal fibrinogen and a thrombin-like fraction derived from snake venom. The adhesive property of this new sealant was tested in animals to study its efficacy in surgeries of colon reanastomoses and sciatic nerve in rats and skin surgery in rabbits (fusiform excision and skin flaps). The results of the tests showed to be promising, and thus, toxicity tests were carried out (determination of LD_{50} in animals, dermal sensitivity tests, mutagenicity assays by the test of Ames *Salmonella*). The toxicity tests were negative which permitted the use of the new fibrin glue in humans. Thus, the objectives of the present work were to evaluate the use of the new adhesive in dermatological surgeries and to verify its effect on healing and on cosmetic results. Eight patients with basal cell carcinoma on the dorsum of the nose were evaluated to date. After excision, these individuals were subjected to autologous skin grafts, the donor areas being the two nasolabial folds bilaterally. On one side of patients' faces, conventional suturing was made, while on the other side fibrin glue was applied. Comparisons were made on the same individuals. Analysis of the results obtained revealed that the length of surgery and bleeding rate were lower on the glued sides than on sutured sides. In addition, 48 h after skin grafting, the surgical wounds were completely sealed having less inflammation than the conventionally sutured sides. No side effects have been observed until now.

O-61Th

MOLECULAR CLONING AND SEQUENCING OF THE VARIABLE REGION GENES OF THE HEAVY AND LIGHT CHAINS OF HUMAN ANTI-TETANUS MONOCLONAL ANTIBODY MAB-G6 WITH HIGH NEUTRALIZING ACTIVITY

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For improvement of serotherapy of tetanus, we previously established six hybrid cell lines producing stably anti-tetanus human monoclonal antibodies (MAbs) with high neutralizing activity. Among them, MAb-G6 which recognizes the middle domain [B] of the tetanus toxin molecule has the highest neutralizing activity of ca. theoretically maximum activity. In this study, we used reverse transcriptase polymerase chain reaction to directly amplify the immunogloblin (IgG) variable region genes from mRNA isolated from the hybridoma producing MAb-G6. Oligonucleotide primers used were as follows; oligonucleotides corresponding to the N-terminal amino acid residues No. 1-10 of human light chain (CL primer) and those to the residues No. 1-7 of human heavy chain (IgG primer) as forward primers and oligonucleotides corresponding to 3' constant regions for the amino acid residues No. 109-116 of human IgG light chain (κ series primer) (since MAb-G6 has a κ light chain) and those to the residues No. 115-121 of human heavy chain (VH series primer) as back primers. The cDNAs subcloned in pBluescript SK were sequenced using a fluorescent labeled primer cycle sequencing kit and an automated DNA sequencer. The analysis of the sequences of the variable regions of the heavy and light chains of MAb-G6 carried out by BLAST search system on Kabat's data base showed that the heavy chain of MAb-G6 belongs to human heavy chain subgroup VH-3 family and the light chain of MAb-G6 is highly similar to kappa light chain subgroup 3 family. The present result will give a basis for producing anti-tetanus recombinant antibody fragments with high neutralizing activity in an appropriate expression system.

O-62Th

TARGETING TO IMMUNE CELLS INCREASES TOXIN IMMUNOGENICITY

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We previously observed that the fusion of a toxic protein to Ig binding proteins such as Protein A from *S. aureus* or its derived ZZ fragment increased immunogenicity of the toxin, *in vivo*. We now show that (i) fusion to ZZ enhanced toxin presentation to a toxin-specific T-cell hybridoma (T1B2), using either A20 B-lymphoma cells or splenocytes as APCs; (ii) this enhancement further increased when the number of fused Ig binding domains varied from 2 with ZZ to 5 with Protein A and (iii) the phenomenon vanished when the fusion protein was preincubated with an excess of free ZZ or when APCs lacked surface Igs, like P388D1 monocytes cells. Therefore, the ZZ-fused toxins are targeted to surface Igs of APCs by their ZZ moiety. To further improve the targeting efficiency, ZZ-toxin conjugates were non covalently complexed to various Igs directed to different cell-surface components of APCs. The resulting complexes were up to 10^3-fold more potent than the free toxin at stimulating T1B2. Also, they elicited both strong T-cell and antibody responses in BALB/c mice, without the need of adjuvant. This simple approach may be of interest to increase immunogenicity of recombinant toxic proteins.

O-63Th

EFFICACY OF TWO DOSING REGIMENS OF AFFINITY PURIFIED, MIXED MONOSPECIFIC CROTALID ANTIVENOM OVINE FAB (CROTAB)

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CroTAb consists of affinity purified ovine Fab capable of neutralizing the toxic effects of North American crotalid venoms. OBJECTIVE: Comparison of the efficacy of two dosage regimens of CroTAb with a validated severity score (SS). METHODS: 30 subjects with worsening minimal to moderate North American crotalid snake envenomation were enrolled in an open-label, randomized, multicenter trial of two dosage schedules of CroTAb. Patients received an IV "initial control" dose of CroTAb. Afterwards, patients were randomized to receive additional CroTAb either as needed (PRN group) or as 2 vial infusions every 6 hrs for three doses (Scheduled group). SS was determined before CroTAb infusion (Baseline), at the end of initial control dose (0 hr), and at 12 hrs. Efficacy was defined as no increase in SS after 0 hr. RESULTS: ANOVA indicated decreases in SS ($p<0.01$) from Baseline for both groups but not between groups at any time point. Fisher's Exact test indicated no difference in odds ratio for SS increase in either group. CONCLUSIONS: Despite documented worsening at enrollment, there was a decrease in mean SS after CroTAb infusion, but there was no difference between mean SS for either dosing regimen.

Group	n	Mean SS (± SD)			0 & 12 hr SS Change	
		Baseline	0 hr	12 hr	# SS ↑	# SS ↓/=
PRN	15	4.80(2.51)	3.33(1.35)	2.53(1.06)	0	15
Scheduled	15	4.00(1.25)	3.07(1.44)	2.40(1.12)	1	14

O-64Th

Bothrops asper MYOTOXIN INHIBITOR PROTEIN (BaMIP): A NOVEL ANTI-TOXIC AND ANTI-PHOSPHOLIPASE A_2 FACTOR FROM THE BLOOD OF *B. asper*

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Envenomations caused by snake bites of *Bothrops asper* (fer-de-lance) are clinically characterized by local tissue damage, hemorrhage, and edema. *B. asper* venom contains basic phospholipase A_2 (PLA_2) myotoxins (I, II, III, and IV) which contribute to muscle necrosis and edema. This study describes the biochemical and pharmacological properties of a *B. asper* myotoxin inhibitor protein (BaMIP) isolated from the blood of the same snake. BaMIP was isolated from crude *B. asper* plasma by affinity chromatography with Sepharose-immobilized myotoxins. Biochemical studies indicated a subunit molecular weight of 23-25 kDa by SDS-PAGE, an isoelectric point of 4, and glycosylation. Gel filtration revealed a molecular weight of 120 kDa, suggesting that BaMIP is an oligomer composed of five 23-25kDa subunits. Functional studies indicated that BaMIP inhibits the PLA_2 activity of the enzymatically active *B. asper* myotoxins I and III, as well as the *in vivo* myotoxicity, *in vitro* cytolytic activity towards cultured endothelial cells, and edema-forming activity of all four myotoxin isoforms (I-IV). The first 63 amino acid residues from the N-terminus of BaMIP share >65% sequence homology with blood PLA_2 inhibitors from *Trimeresurus flavoviridis* and *Agkistrodon blomhoffii siniticus*. BaMIP and these inhibitors contain carbohydrate recognition domains (CRDs) present in human and rabbit cellular PLA_2 receptors, suggesting a common domain evolution among snake plasma PLA_2 inhibitors and mammalian PLA_2 receptors. Despite this homology, this is the first description of a natural anti-myotoxic factor from snake blood.

O-65Th

ENVENOMATION BY *Tityus stigmurus* IN THE METROPOLITAN REGION OF BAHIA, BRAZIL

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The accidents caused by scorpions represent about 30% of the total number of human envenoming in Bahia, Brazil. The present investigation is a descriptive study about the clinical aspects of the accidents caused by *Tityus stigmurus* in the metropolitan area of Salvador, Bahia, Brazil. We analysed 94 confirmed cases treated by the Information Centre of Antivenom (CIAVE) from 1982 to 1995. Envenomation by *T. stigmurus* is mainly characterized by: local disturbance manifestations: pain (94.4%), dormancy (28.9%), edema (17.8%), erythema (17.8%) and paresthesia (15.6%). General manifestations such as headache (4.4%), vomiting (4.4%) and sudoresis (3.3%) were observed with less frequency. Most of the envenomation cases were mild (94.0%) and all evolved to cure. The high frequency of mild cases may also indicate the benignity of the *T. stigmurus* accidents in this region. Sorotherapy was used in 8.0% of the patients and none of the cases resulted in death. The absence of the lethality and the high frequency of mild cases suggest that the treatment with aracnidic antivenom is not absolute indicative for children above seven years old as reported by Rodrigues *et al.* (1993).

O-66Th

HORSE IgG ISOTYPES PRESENT IN ANTIVENOMS PRODUCED BY INSTITUTES CLODOMIRO PICADO (CR) AND BUTANTAN (BR)

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The polyvalent serum of Instituto Clodomiro Picado (ICP) and anti-bothropic serum of Instituto Butantan (IB) have been evaluated and compared regarding their neutralising ability, IgG subclasses content and neutralisation of lethality by IgG(T) and IgGa. Different amounts of ICP or IB serum were mixed with $3LD_{50}$ *Bothrops asper* (LD_{50}=77.5 µg/20 g mouse) or *Bothrops jararaca* (LD_{50}=33.0 µg/20g mouse) venom, respectively, and injected i.p. into three groups of six Swiss mice. The survival recorded after 48 hours was used for calculating the ED_{50} (20 g mouse) by probit analysis. The ED_{50} for ICP serum was 114.6 µl/mg venom (CL95% 74.8-141.6) while that for IB serum was 90.1 µl/mg venom (CL95% 70.5-109.6). The IgGa and IgG(T) isotypes were then isolated from both antivenoms by chromatography on protein A-Sepharose followed by anti-IgG(T) mAb-Sepharose (LO-HoGT-1). The ED_{50} (mg/mg venom) for IgG(T) isolated from ICP serum was 4.6 (CL 95% 4.0-5.3) while that isolated from IB serum was 4.1 (CL95% 3.7-4.4). The ED_{50} for IgGa from ICP serum was > 13 and from IB serum was 13.3 (CL95% 9.7-18.7). No significant difference was detected on ELISA antibody titres of the different IgG subclasses to *B. jararaca* (IB serum) or *B. asper* venom (ICP serum). IgG(T) had the highest titre, followed by IgGa, IgGb and IgGc. These results suggest that both sera have the same IgG antivenom profile and for neutralisation of lethality IgG(T) is the most important isotype, followed by IgGa. We are currently investigating the ability of IgG(T) and IgGa to neutralise other effects as haemorrhage, myonecrosis, oedema and coagulant activity.

Supported by FAPESP, Fundação Butantan and European Community (CII*CT94-0043)

O-67Th

Thalassophryne nattereri FISH VENOM: BIOCHEMICAL CHARACTERIZATION AND NEUTRALIZATION OF TOXIC ACTIVITIES

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Freshly collected or frozen *T. nattereri* venom injected i.d. in the foot pad of mice induced local edema followed by necrosis. Subcutaneous injection of the venom induced systemic effects consisting of jerking motions, paralysis of hind limbs, erection of the hair, rotational movements and after 6 hr. violent convulsions followed by death. Necropsy showed hyperemia of the lung and small intestine. Other organs seemed normal. The venom showed distinct myotoxic and hemolytic activity, low levels of proteolytic activity and no phospholipase A_2 activity. SDS-PAGE analysis of the venom showed 17 components with the major bands located around 19,000 mol.wt. Isoelectric focusing of the venom showed mainly basic proteins. Fractionation of the venom by gel chromatography or cation exchange chromatography resulted in a few distinct peaks but in both situations the biological activities were located in only one of the peaks. Heating of the venom at 56° for 60 min destroyed its biological activities. All venom toxic activities except edema were completely neutralized by *in vitro* incubation with anti-*T. nattereri* venom serum.

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O-68Th

COBRA ANTIVENOM
EVALUATION ANTICOBRA ANTISERUM PRODUCED IN VIETNAM

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Venomous snakes and their victims are getting interest of many countries in the world , especially those in tropical areas , such as Vietnam. Since 1992 , the project " Research on production of Cobra antivenom (CAV)" has been researched in Vietnam . The authors have successfully produced CAV , meeting the National and International Standards .

Venoms from naja naja kaouthia and naja naja atra were immobilized on CNBr - activated Sepharose 4B . Serum from horse immunized with both venoms was subumitted for specific antibody isolation . Fab and Fab' - s - biotin were obtained from the immuno-affinity purified antibodies . Fab was covalently conjugated to polystyrene microplate . The ELISA system was used as detecting venom .
Calibration curves using separately naja naja kaouthia and naja naja atra venoms manifested a same slope from 1 ng / ml to 1µg / ml venom concentration - Probably the high proportion of the neurotoxin and cardiotoxin and their homology explain these results for these two ophidian species .
Up to now , there have been 36 victims of cobra snakes being rescued by CAV produced at Choray hospital .

O-69Th

A QUANTITATIVE COMPARISON ON THE REFINEMENT OF HORSE ANTIVENOM BY SALT FRACTIONATION AND ION-EXCHANGE CHROMATOGRAPHY

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A quantitative comparison was made on the fractionation of pepsin-digested horse antivenoms by ammonium sulfate (AS) fractional precipitation and ion-exchange chromatography on Q-Sepharose. In the precipitation process, pepsin digested horse anti-*Naja kaouthia* serum was precipitated by 30% saturated AS followed by 50% saturated AS. The recovery of antibody activity (as measured by an ELISA against the cobra postsynaptic neurotoxin 3) from the 30-50% saturated AS precipitate was 53% with a 1.93 fold purification. For the chromatographic process, the behavior of the horse antitoxin antibody and its $F(ab')_2$ fragments were first studied. The pepsin digested horse serum was then desalted on a Biogel P-2 column followed by chromatography on Q-Sepharose using a linear gradient (20 mM Tris-HCl pH 8.0 containing 0.0 to 0.5 M NaCl). A peak containing primarily the $F(ab')_2$ antibody could be obtained. This peak constituted 73% of the total antivenom activity with 2.08 fold purification. The total recovery of antibody activity by the chromatographic process was 90%. The yield of antibody activity was about 2 fold higher than that reported previously with other fractionation procedures. The implications of these results for the refining of horse therapeutic antivenoms are discussed.

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O-70Th

COMPARED PHARMACOKINETIC AND EFFICACY OF $F(ab')_2$ AND Fab IN EXPERIMENTAL VIPER ENVENOMATIONS

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Specific $F(ab')_2$ fragments are widely used for the preparation of antivenoms since many years. More recently, anti-digoxin Fab proved to be very efficient in the treatment of digoxin intoxication by reducing the volume of distribution of the drug and by accelerating its elimination. Some investigators therefore suggested to use sheep Fab instead of horse $F(ab')_2$ for the immunotherapeutic treatment of snakebite envenomations.

In this investigation, we first compared the effect of the same doses of $F(ab')_2$ and Fab prepared from the same hyperimmunized horse serum on the pharmacokinetic parameters of *Vipera aspis* venom components in experimentally envenomed rabbits. We observed that $F(ab')_2$ were more efficient than Fab to cause redistribution in the vascular space of the venom components and to neutralize them *in vivo*. These observations are in agreement with the pharmacokinetic parameters of the two types of fragments: Fab being more widely distributed and more rapidly cleared than $F(ab')_2$. They also agree with clinical trials of sheep antivenom performed in the case of viper bites in Sweden, which indicated that Fab induced a neutralization of a shorter duration than that observed with $F(ab')_2$ and that a continuous infusion of Fab instead of a bolus injection is necessary to obtain an effective neutralization of the venom.

The determination of the pharmacokinetic parameters of immunocomplexes made of venom components associated with either $F(ab')_2$ or Fab indicated that Fab-venom complexes are eliminated slowerly than free Fab and that $F(ab')_2$-venom complexes are eliminated with a faster rate than free $F(ab')_2$.

This experimental investigation indicates that Fab are less effective than $F(ab')_2$ to neutralize intoxications with large molecular weight components such as that of viper venom, while the reverse is observed for small molecule as digoxin. The possible explanation for these differences in the efficacy of the Fab might be due to the fact that the low molecular weight of digoxin-Fab complexes allows them to be rapidly eliminated by renal route, whereas the high molecular weight of Fab-venom complexes (>100 kDa) exceed the glomerular filtration threshold.

O-71Fr

Functional mapping of α-dendrotoxin, a blocker of voltage dependent K^+ channels.

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α-dendrotoxin (DTX) is a 59 amino acids peptide, structurally related to Kunitz-type proteases inhibitors, that is isolated from the venom of the green mamba *Dendroaspis angusticeps*. It was first described as enhancing acetylcholine release at neuromuscular junctions. Further studies have shown that it blocks numerous voltage-dependent, rapidly activating, slowly inactivating neuronal potassium currents that correspond to Shaker-type channels (Kv1). Studies using K^+ channels produced by the expression in heterologous systems of the α-subunits of these channels have revealed that DTX binds to the loop between the transmembrane spanning segments S5 and S6, a region that comprises the ion pore.

In order to identify the site by wich DTX binds to these channels, we produced by genetic or chemical means, 25 analogs of the toxin in which one residue has been replaced by an alanine. With the exception of one analog, circular dichroism indicated that these substitutions did not perturb the global structure of the toxin. For measuring the functional impact of the substitutions, we studied the binding of the analogs to rat brain potassium channels, using a competition assay with iodinated DTX.

Among the 25 residues tested, six were found to be implicated in the binding of DTX to rat brain K^+ channels. The most important are K5 and L9 whose substitutions induce a 1000 fold decrease of affinity, followed by R4, L6 and I8 whose substitution induces a 10-100 fold decrease in affinity and R3 whose substitution induces a 5-10 fold decrease in affinity. All these residues form a cluster, the center of which is occupied by K5. Further substitutions of this residue (K5Nle and K5Orn) indicated that both the positive charge of the ε-NH_2 and its distance from the α-carbon are important for the α-dendrotoxin binding to potassium channels.

The functional map of α-dendrotoxin can be now wcompared to those of other potassium channels blocking peptides.

O-72Fr

Processing of Pro-Tumour Necrosis Factor-α by venom metalloproteinases and their inhibition

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Venom-induced necrosis is a common local debilitating sequel of bites by many vipers, frequently resulting in severe permanent scarring and deformity. Antivenoms are not effective under these circumstances unless administered within a few minutes of the bite; this is unlikely to occur in the rural tropics where most victims take a long time to reach medical care. We have shown that two venom zinc metalloproteinases (Jararhagin from *Bothrops jararaca* venom and EcH-II from *Echis pyramidum leakeyi* venom) successfully cleaved the recombinant GST-TNF-α substrate to form biologically active TNF-α which was shown to be neutralised by ovine TNF-α Fab antibodies resulting in a reduction of venom-induced necrosis in mice when injected intravenously or intradermally both before and after intradermal injections of *E.p.leakeyi* venom. A peptidomimetic (POL 647) was also found to inhibit EcH-II and thus prevent the processing of the TNF precursor using a TNF-α sensitive cell culture assay and electrophoresis. These observations demonstrate the possible importance of TNF-α in the development of the resulting necrotic lesion. This has led to the hypothesis that increased levels of venom metalloproteinases following snake bite preferentially release active TNF-α inducing production of endogenous matrix metalloproteinases (MMPs) which generate a positive feed-back mechanism resulting in repeated cleavage of pro-TNF-α. The processing of pro-TNF *in vitro* and the liberation of this cytokine by venom metalloproteinases may explain the clinical observations that treatment of local necrosis following snake bite by antivenom is only minimally successful.

O-73Fr

EXPRESSION OF JARARHAGIN DISINTEGRIN DOMAIN IN *Escherichia coli*

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Jararhagin, a long-chained haemorrhagin from *Bothrops jararaca* venom, is a multidomain enzyme belonging to the metalloproteinase/disintegrin protein family. The haemorrhagic activity is associated with the metalloproteinase domain while the disintegrin domain has been correlated with binding to platelets and inhibition of collagen-induced platelet aggregation. The expression of Jararhagin, and particularly its disintegrin domain, is therefore important to elucidate the molecular basis of the haemorrhagic toxins and their possible role as disintegrin-like components. The cDNA fragment coding for Jararhagin disintegrin plus cysteine-rich domains was amplified by PCR from the BJD4 clone, originally cloned in pBluescript plasmids (Paine *et al.*, *J. biol. Chem* 267: 22869, 1992).The resultant PCR product was then cloned into pET 32a vector using the restriction sites *SacI* and *NotI*. Among the positive clones the JD9 was used to transform *E. coli* AD494 and BL21 strains. The induction of expression was carried out by addition of 1mM IPTG on log phase cultures (OD=.500) for four hours. The thioredoxin-disintegrin fusion protein was recovered from the soluble extract of the cells; yields varied from 20 mg/l (AD 494) to 50 mg/l culture (BL21). The fusion protein was then isolated using poli-histidine binding resin which resulted in a main band of 45 kDa recognised by anti-native Jararhagin antibodies. Antibodies raised in rabbits against the fusion protein had high ELISA titres against native Jararhagin and also detected a band of approximately 52 kDa on the Western blots of whole *B. jararaca* venom. Treatment of the fusion protein with enterokinase followed by further capture of the enzyme and the histidine-containing thioredoxin fragment resulted in a main band of 30 kDa; this was capable of partially inhibiting collagen-induced platelet aggregation. We are currently investigating the activity of this fragment on venom-induced local effects and venom neutralisation by anti-disintegrin antibodies.

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O-74Fr

BIOCHEMICAL CHARACTERIZATION, cDNA CLONING AND EXPRESSION OF THE FIBRINOLYTIC ENZYME, LEBETASE FROM *VIPERA LEBETINA* VENOM

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Lebetase, a strong fibrinolytic enzyme, was isolated from the venom of *Vipera lebetina*. It is metalloproteinase which contains one mole zinc and one mole calcium per molecule. Lebetase cleaves fibrinogen and fibrin directly, without plasminogen activation. The fibrin(ogen) Aα-chain is cleaved first, followed by cleavage of the Bβ-chain. *V. lebetina* venom contains several isoforms of lebetase. Two lebetase fractions (pI 5.0, pI 5.3) were separated. The mol. weights of the cleavage products of fibrin and fibrinogen produced by two different lebetase isoforms are identical. The antibodies against these fractions reacted only with the fibrinolytic enzyme in *V. lebetina* venom as demonstrated by Western blotting.

Lebetase cDNA (Le-3) was isolated from the *Vipera lebetina* venom gland cDNA library. The cDNA sequence with 2011 basepairs encodes an open reading frame of 478 amino acids which includes an 18 amino acid signal peptide, plus an 175 amino acid segment of zymogen-like propeptide, a mature protein of 204 amino acids, a spacer of 18 amino acids and a disintegrin-like peptide of 63 amino acids. The lebetase gene was subcloned into a baculovirus expression vector pFastBac1 downstream of the polyhedrin promoter (BamHI-XhoI) using standard methods.

Upon infection of SF9 cells, a 23 kDa band showed up on SDS-PAGE gel stained with Coomassie BB. Western blot analysis of infected cells showed the presence of a 23 kDa band in SF9 cells after 72 hr of incubation. The SF9 cells had fibrinolytic activity on fibrin plate which was also highest after 72 hrs of infection.

O-75Fr

GENE SEQUENCE OF AN AUSTRALIAN TICK NEUROTOXIN.

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Envenomation by the Australian paralysis tick *Ixodes holocyclus* results in a severe toxicosis affecting thousands of domestic pets and livestock each year. Current antivenom treatment is expensive and is only effective in the early stages of paralysis. This disorder is characterised by a rapidly ascending paralysis and is due to the presence of neurotoxins in tick salivary glands.

Recently three neurotoxins (HT-1, HT-2 and HT-3) were identified by synaptosome binding experiments and isolated by HPLC. The three neurotoxins were N-terminally blocked but internal peptide sequence was obtained for HT-1 following tryptic digestion. Using PCR primers derived from the partial amino acid data, the gene for HT-1 was isolated and sequenced. The gene codes for a 50 amino acid polypeptide containing 8 cysteines and has significant homology to other arachnid neurotoxins from scorpions and spiders. These homologies of sequence, cysteine-cysteine motifs and signal peptide will be discussed. The gene has been successfully expressed as a fusion protein which shows antigenic properties similar to the native toxin.

O-76Fr

VENOMS: A FULLY INTERACTIVE DATABASE ON VENOMOUS ANIMALS AND THEIR VENOMS

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VENOMS is a new database offering a unique variety of information in the field of venomous animals and their venoms. It contains a complete list of all amino acid sequences of venomous animals proteins that have been described so far. This registry contains some 800 toxins and enzymes with as much structural and functional information as possible for each of them, together with direct links to the animals description and bibliographical references.

A complete list of more than 800 venomous snakes with full descriptions including complete zoological classification according to the latest systematic nomenclature, actual scientific name, common names (in English, French and German), synonyms, geographical range with illustrated colour geographic maps, and many professional colour pictures of the animals is also included.

The database is further incremented with an extensive bibliography containing more than 60'000 references covering all publications in the field of venomous animals, including features such as title, authors, abstract, source, year of publication and keywords.

VENOMS is available on CD-ROM and can be accessed by any microcomputer running under a Windows operating system (version 3.1 or later). The multimedia based interface is made easy through a specifically developed software that allows direct access to information through user friendly navigation buttons, special indexes as well as full text search facilities.

O-77Fr

FINGERPRINTING OF CRUDE VENOMS BY MASS SPECTROMETRY: ZOOLOGICAL AND MOLECULAR CONSIDERATIONS

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Electrospray mass spectrometry (ES-MS) plays an important role in the analysis and characterisation of intact peptides and proteins allowing accurate molecular weight determination. A reversed phase HPLC (high pressure liquid chromatography) system can be directly coupled to the MS instrument (On-line LC-ES-MS), allowing direct analysis of the elution profile of complex mixtures such as crude extracts. We have analysed crude venoms of different origins on a microbore reversed-phase HPLC system (20 µg injected). The absorbance was followed by UV detection, the eluate was split, and a portion was collected in fractions for further structural and functional studies. The other portion of the eluate was directly introduced into the mass spectrometer and further followed by Total Ion Current (TIC) at the detector end of the MS instrument. The mass spectra corresponding to each signal were averaged, allowing molecular weight determination of various biopolymers with a mass accuracy of ±1 Da up to 30 kDa. The highly specific toxin mass maps that could thus be obtained for each sample were used as fingerprints for a definite zoological classification of each animal. Furthermore, the different measured molecular weights were compared in a database with those of previously described toxins, leading to structural and functional information on the individual venom components and to the identification of new toxins. An appropriate combination of enzymatic digestions and reductions followed by LC-ES-MS and MS-MS analysis was used to fully characterise the amino acid sequence, disulfide bridges and other possible post-translational modifications of one of these previously undescribed toxins.

Our results demonstrate that this powerful method can be used from crude extracts to molecular characterisation allowing simultaneous zoological classification of a venomous animals and structural investigations of its individual toxins.

O-78Fr

ACETYLCHOLINESTERASE FROM *ELAPIDAE* VENOM: IDENTIFICATION OF A NEW ALTERNATIVE EXON AND OF TWO RESIDUES IMPLICATED IN THE PERIPHERAL SITE

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Acetylcholinesterase (AChE) is a key enzyme in the regulation of synaptic cholinergic transmission. It is also present in non-cholinergic contexts where its role is not clearly defined. This is the case in *Elapidae* venoms which are very rich sources of this enzyme: they contain soluble AChE monomers; these enzymes present variable sensitivity to peripheral site inhibitors, in particular fasciculin 2 from *Dendroaspis* venom. To analyze these properties at the molecular level, we cloned the cDNA encoding AChE from *B. fasciatus* venom. The deduced C-terminus peptide is short (15 residues) and does not show any similarity with the C-terminal sequence encoded by alternative exons T or H in other vertebrates. This peptide is encoded by a new exon, which is used alternatively with a T exon.

We also identified two major differences localized in the peripheral site: *B. fasciatus* AChE has a methionine in position 70 and a lysine in position 285, instead of a tyrosine and an acidic residue in *Torpedo* AChE. The residue Y70 is clearly part of the peripheral site but the residue 285 has never been studied in this connection. We restored a "*Torpedo*-like" situtation by modifying by site-directed mutagenesis the *B. fasciatus* AChE with the mutants M70Y and K285D, and the double mutant M70Y/K285D. Each point mutation increased tenfold the sensitivity of *B. fasciatus* AChE to fasciculin and these effects were combined in the double mutant.

O-79Fr

INFLAMMATORY HOST RESPONSE TO *Bothrops atrox* VENOM

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Human beings show different susceptibilities to the toxic activities of snake venom,this variability being attributed to either the intrinsic venom composition or to genetic variability of he victims,or both.Several isogenic strains of mice injected with the same sample of *Bothrops atrox* venom showed different susceptibilities to its toxic activities.C57Bl/6 (LD_{50} 86ug/20g) being more susceptible than C3H/Pas strain (LD_{50} 140ug/20).In BALB/c mice an intermediate susceptible strain ,the pattern of the systemic release of some cytokines upon ip injection of 50 ug of the venom is the following:IL-6, IFN-γ and Il-10 attained the maximal release of some cytokines at 4 hs while the peaks for TNF-α, NO and the second peak for INF-γ were attained at 18,24 and 24 hs respectively. Supernatants of spleen cells obtained from mice primed ip with 50µg,3 hs before cell harvesting and cultivated in absence or presence of 250 ng of venom released IL-6, IL-10, and IFNγ reaching the peak at 48 hs of incubation Resident macrophages pre-stimulated *in vivo* and challenged *in vitro* with 250ng of venom or LPS release IL-6,IL-10 and IFN-γ whereas under the same conditions the cells were incapable of producing TNF-α and NO. Inflammatory macrophages pre-stimulated *in vivo* with thioglycollate and venom and challenged *in vitro* with 250 ng of venom showed diminished production of TNF-α and NO as compared to the the controls that were only stimulated in vitro either by venom or LPS. Subcutaneous injections of venom resulted in leucocyte accumulation, oedema, haemorrhage and necrosis which increased from 30 min to 12 hs. The high intensity of lesions coincided with the maximal release of TNF-α, IFN -γ and NO. Using a combination of gel filtration, ionic exchange and a metallo protease inhibitor affinity chromatography the enriched fraction obtained with hemorrhage activity, analysed by SDS-PAGE is resolved in two bands of 30 and 60 kDa.Some monoclonal antibodies raised against whole venom were able to inhibit hemorrhagic activity.Results of western blot analysis indicated that these monoclonals recognize proteins with the same MW as the purified protein(s) endowed with inducing hemorrhage activity.

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O-80Fr

CENTRAL AND PERIPHERAL MECHANISMS OF THE ANALGESIC EFFECT OF *Crotalus durissus terrificus* (Cdt) SNAKE VENOM

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The antinociceptive property of venoms of some snakes species, including Cdt venom, have been demonstrated in human beings and in experimental animal models. The Cdt venom causes a long-lasting antinociception in mice, as measured by the hot plate test, but not in the tail flick test. This response indicates that in the Central Nervous System, the action of the venom is not due to inhibition of some specific spinal reflex elicited by stimulation of nociceptive pathways in the tail flick model, but depends on inhibition/stimulation of undetermined upper central centers. Pharmacological studies demonstrate that the effect of the venom is mediated by opioid and muscarinic receptors. Long-term treatment with Cdt venom induces the development of tolerance to its antinociceptive effect. Crossed-tolerance to the action of morphine is not observed. On the other hand, using the same experimental conditions, the venom does not induce physical dependence. In addition to this central effect, the Cdt venom causes antinociception in the rat paw carrageenin-induced hyperalgesia assay, a model of inflammatory hyperalgesia. Using naloxone as an opiod antagonist, it could be demonstrated that the venom activity is partially mediated by peripheral and local opioid receptors. It was also shown that the antinociceptive effect of Cdt venom is not due to alterations in the motor or coordination activities of the animals.

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O-81Fr

RECURRENCE OF COAGULOPATHY FOLLOWING NORTH AMERICAN PIT VIPER ENVENOMATION

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Introduction: Case reports suggest that following envenomation by North American pit vipers coagulation abnormalities may recur after an initial resolution of coagulopathy. This study describes the course of coagulopathy following North American pit viper envenomation. Methods: A database of 354 consecutive cases of snake bite injury reported to a certified regional poison center between 1986 and 1992 were reviewed for platelet count (PLT), prothrombin time (PT) and fibrinogen level (FIB). All cases of coagulopathy (PLT<150,000/mm^3, PT>normal, and/or FIB<150 mg/dL) were examined for duration and recurrence of these abnormalities. Recurrence was defined as any abnormal PLT, PT or FIB value occurring after the initial normalization of these parameters. Results: There were 137 cases (38%) having coagulation test abnormalities during hospitalization. Overall, 27 involved thrombocytopenia alone, 65 involved abnormalities of the coagulation cascade (PT or FIB) alone and 45 had both. Recurrence was evident in 18 cases (13%) with an average onset time of 32 ± 18 hours from envenomation (range 12 to 63 hours). Six cases had a recurrence of thrombocytopenia (36,000 to 145,000), 8 involved abnormalities of the coagulation cascade (PT: 14.5 to 15.7 sec; FIB: <15 to 139 mg/dL) and 4 were a combination of both. Antivenin (Crotalidae) Polyvalent [Wyeth] was used in 13 of the 18 recurrent cases with an average of 15 ± 10 vials administered. Of 137 cases of coagulopathy, 112 (82%) received Wyeth antivenin and 13 (12%) developed recurrence. Conclusion. Patients with North American pit viper envenomation can have a recurrence of coagulopathy. The recurrence of coagulopathy in patients can manifest irrespective of treatment with Antivenin (Crotalidae) Polyvalent [Wyeth].

O-82Fr

Characterisation and biological activity of 3-alkylpyridinium compounds from the marine sponge *Reniera sarai*.

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We isolated 3-alkylpyridinium polymers (poly-APS) from the marine sponge *R. sarai*. Two kinds of poly-APS were detected by NMR and MALDI-TOF spectrometry that are composed of the same repeating units but differs in terms of molecular mass. Large poly-APS (18900 Da) are composed of 100 3-octylpyridinium units which are head to tail polymerised, while smaller poly-APS (5520 Da) are polymerised from 29 monomeric units. We found that poly-APS in aqueous solutions form large non-covalently bound aggregates with a mean hydrodynamic radius of 23 nm. The aggregation depends on poly-APS concentration. Poly-APS strongly inhibit cholinesterases from different sources but are ineffective against enzymes like trypsin or alkaline phosphatase. The inhibition kinetics is rather complex with a clear time-dependent irreversible component. We studied interaction of the enzyme with poly-APS by means of CD and flourescence spectroscopy. We may conclude that irreversible inhibition of the enzyme is not due to the simple enzyme denaturation but rather to the formation of large complexes between poly-APS and AChE which completely blocks the enzyme. Poly-APS are strongly hemolytic. Their activity towards red blood cells could be compared to the cell permeabilization by cationic surfactants like cetylpyridinium chloride. The hemolysis induced by poly-APS could be partially blocked by compounds like PEG-4000 or completely prevented by cations like Hg^{2+} (μM conc. range) or Zn^{2+} (1 mM conc.). Poly-APS also possess cytotoxic activity against different cell lines, but show little specificity against transformed cells. "In vivo" poly-APS cause transitory cessation of breathing, bradycardia and hypotension which may eventually lead to the death of experimental animal. The estimated i.v. LD_{50} for rats is about 2 mg/kg.

O-83Fr

NEUROTOXIC PHOSPHOLIPASE A_2-INDUCED EPILEPTIC SYNDROME: A COMPLEX MODEL

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After intracerebral injection, some toxic secreted phospholipases A_2 ($sPLA_2$) can induce an epileptic syndrome. The bases for such an effect are currently ill-known. The role of phospholipid hydrolysis is probable although not proven yet. Binding to the recently described neuronal receptors may also be important. The scarce data found in the literature strongly suggest a high variability of the epileptogenic properties of these enzymes. Moreover, because of their high affinity for neuronal receptors, snake venom neurotoxic $sPLA_2$ could be very valuable pharmacological tools to investigate the role of endogenous $sPLA_2$ in other models of epilepsy. We thus undertook the study in the rat of the neurotoxic and epileptogenic properties of two toxins, paradoxin (PDX) and crotoxin (CTX) that may share similar receptors. Related toxic enzymes (ammodytoxin A , ATX, and CTX basic subunit CB) and nontoxic $sPLA_2$ from pancreas were also tested. They could effectively be differentiated based on two criteria, the importance of cortically-recorded seizures (E) and convulsions (C). We thus propose to classify $sPLA_2$s into four groups depending on their epileptogenic properties: E-C- (PDX, pancreatic enzyme), E+C+ (CTX, CB), E-C+ (ATX) and E+C-. Moreover, the results obtained with chemically-inhibited CB strongly suggested that phospholipid hydrolysis is of paramount importance for this $sPLA_2$ to trigger seizures and convulsions. Besides a slightly different catalytic activity, the reasons that could explain the large difference between PDX and CTX epileptogenic properties are not known as yet and would require further studies.

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O-84Fr

TOXIC EFFECTS OF CATNIP (*NEPETA CATARIA*) EXPOSURE DURING EMBRIOGENETIC PERIOD IN MICE

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Catnip or catmint (*Nepeta cataria*) is perennial herb belonging to the mint family Labiatae. It is an hallucinogenic plant that has been extensively used in toy for pets as well as for human abusers. Cats and other wild animals react to catnip with behaviors directed toward reward. Man also experiences pleasure when smoke the dry leaves or the extract of catnip. The toxin present in this plant is an essential oil, the nepectalone, whose chemical structure is similar to some parts of marijuana and LSD. The present study was undertaken to investigate the effects of prenatal exposure to catnip on some reproductive parameters of mice and on physical development of their offspring. Dried catnip leaves were obtained from a commercially available product previously tested in cats (Super Catnip, Four Paws Products Ltda), ground and mixed with the basal mouse diet at 10% (w/w) concentration and administered to the experimental mothers from 6 to 18 day of pregnancy. Animals of control group received only the basal diet during the same period of gestation. Results showed a decrease maternal body weight at 18 days of pregnancy and a reduction of fetal and placental weights in animals exposed to catnip in relation to controls. In addition, no differences were observed between the number of living and dead fetuses, in the number of resorptions and in the ratio of maternal body weight/ fetuses weight plus placental weight of both groups. In relation to offspring development, catnip prenatal exposure reduced the body weight of male and female pups at birth while only female showed a decrease in this parameter at 7 days of lactation. The female eye, ear and vaginal openings and male testis descent treated with the plant were delayed in relation to control group. These results suggest that catnip exposure during gestation 1) impaired intrauterine growth of fetuses probably by an action at placental level; 2) did not induced abortions/resorptions or embrioletality; 3) changed the time course of some physical landmarks in female offspring; 4) delayed male and female puberty.Financial support: CNPq- PIBIC and FAPESP.

12th WORLD CONGRESS IST

O-85Fr

STRUCTURE AND TOXIN CONTENTS OF SALIVARY GLANDS OF HUMAN BODY LICE

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Although millions of persons worldwide are injected each year with salivary toxins of human body lice (Pediculus humanus humanus), there is little information about the structure of the glands or of their contents that cause vasodilatory and other effects. Both the reniform glands and the U-shaped glands were observed to possess cells of a unique structure that surround the salivary duct. Electrophoretic analysis showed the quality of proteins in the two kinds of glands to substantially differ. The saliva on both unfed and recently fed lice contains an erythema-inducing. Immunological analysis using sera from hosts heavily fed on by lice reacted more strongly with native salivary proteins than it did with denatured antigens.

O-86Fr

Immunosuppressive Effects of Wasp Venom : Blood Melanization Activity and Cellular Encapsulation.

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Selective removal of venom immediately after its natural injection into embryonic tissue results in a delayed death and cellular encapsulation of the parasite larva in its juvenile host. This removal of venom also results in significantly less suppression of the host blood's production of brown/black melanins, compared to observed when the venom is fully present. However, this change did not arise through altered expression of the major polydnavirus transcript, which was expressed at similar levels in both hosts that were venom-deficient and hosts that received a full complement of persistent venom. The presence of naturally injected venom and polydnavirus, in the selective absence of a parasite larva, results in the hemolymph melanization process being shifted toward pathways that produce yellow/red melanins. Finally, the presence of a normal complement of all three components (venom, polydnavirus and parasite larva) has no apparent effect to block host embyronic melanization, but does prevent visible production of either brown/black eumelanin-like products or yellow/red pheomelanin-like products by the host larva. These results demonstrate that the fully-present, naturally-injected venom has effects on both parasite escape from encapsulation and host melanization reactions. Application of these indices indicated that most of the naturally occurring pseudoparasitized hosts observed in the laboratory colony arise due to the presence of a full complement of venom and polydnavirus, and the absence or death of a parasite egg.

O-87Fr

Effects of Scorpion Antivenom on Hemodynamic Pathophysiology in Dogs Injected with Scorpion Venom.

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Objective: In dogs, scorpion venom (V) cause an immediate increase in cardiac output (CO) and blood pressure (BP) that decline later below baseline value. We tested the hypothesis that antivenom (AV) given simultaneously with the V may cancel its effect while administration of AV after CO and BP decline, does not affect their decrease.

Methods: 22 anesthetized, mechanically ventilated (constant minute volume, no O_2 supplement) and instrumented dogs. 0.1 mg/kg venom of *Leiurus quinquestriatus* was given IV. Measurements of BP, CO, heart rate (HR) pulmonary artery pressure (PAP) and arterial gas tensions were taken before and at 5, 15, 30, 60, 120 and 180 min. following V injection. 5 of the dogs were given V alone. 5 dogs were given 6 ml of AV simultaneously with V. 7 dogs received 6 ml AV at 15-20 min. after V injection, immediately after fall in CO. 5 dogs received 6 mg AV 60 min. after V injection.

Results: V alone caused a decrease in HR from 182 ±13 to 117 ±14 at 180 min. $p<0.01$. BP and CO initial increased from 129 ±22 to 187 ±19 torr, and from 5.0 ±1.1 to 10.8 ±2.7 L/M $p<0.01$ respectively, between baseline and 5 min. CO and BP declined later to 2.4 ±0.98 and 69+15 $p<0.01$ respectively. PAP showed same pattern of changes. V caused also decrease in pH, HCO_3 and PaO_2 and elevated $PaCO_2$. AV given simultaneously with V cancelled all V effects. AV given at 15 and 60 min. did not reverse CO, BP, PAP and HCO_3 decline. HR, PaO_2, pH and $PaCO_2$ however improved considerably in dogs treated by AV compared with dogs which were given V alone.

Conclusion: In dogs injected with scorpion venom deterioration of hemodynamics cannot be reversed when AV is given after CO and BP decline. However, these dogs showed improvement in HR and respiration. These improvements are probably related to anticholinergic effect of the antivenom.

O-88Fr

CHARACTERIZATION OF A COMPLEMENT-DEPENDENT HEMOLYSIN FROM *Botrops atrox* VENOM

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Snake venoms contain components endowed with hemolytic activity. Some act directly on the erythrocyte cell membrane whereas others generate, in the fluid phase from extracellular added or secreted substrates, molecules capable of lysing the cells. Included among the formers are certain small peptides and among the laters the glycoproteins cobra venom factor (CoF) and phospholipase A_2 (PLA_2). CoF organizes a hybrid C3 convertase by biding to the C factor B which constructs the potentially lytic complex C5b-C59; PLA_2 generates lysolecithin by cleaving lecithins. This communication shows that human erythrocytes pre-treated with *B. atrox* but not with *Crotalus durissus terrifucus, Lachesis muta or Micrurus coralinus* were lysed, in a dose-depedent fashion, by C-sufficient autologous serum. The hemolysis was inhibited when C factor B was inactivating by heating the serum at 50 °C for 45 min or by blocking the C alternative pathway C3 convertase assembly by Mg^{+2} chelation with EGTA. The *B. atrox* venom component mediator of this C-dependent hemolysis was purified submiting samples of crude venom to chromatographies on Sephacryl S 100 and DEAE cellulose. The hemolytic activity was retained in a 14-16 kDa protein. This molecule may transform human erythrocytes into activators of the autologous C-alternative pathway problably by rendering the CR1, DAF, CD59 or other C regulatory molecules unable to accomplich their activities.

Supported: FENORTE

ABSTRACTS
Poster (P)

12th WORLD CONGRESS IST

P-001Mo

MAPPING OF THE FUNCTIONAL SITES OF a-CONOTOXINS

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α-conotoxins are small peptides found in sea snail venoms. They block various subtypes of nicotinic acetylcholine receptors (n-AcChoR). The a-conotoxin GI, isolated from *Conus geographus* binds with high affinity (Kd = 45 nM) to the peripheral receptor from *Torpedo marmorata.*, whereas it binds with a low affinity (Ki = 150 μM) to the recombinant chimaeric neuronal-type a7-5HT3 receptor, expressed in HEK cells. In contrast, the a-conotoxin ImI binds with a higher affinity (Ki = 4μM) to the neuronal subtype as compared to the peripheral receptor (Ki = 200 μM). With the view to understanding the molecular basis associated with this differential specificity, we investigated the functional sites by which these toxins interact with the two receptors.
A systematic alanine-scanning carried out with the a-conotoxin GI, revealed the important binding role of Pro-5 and Tyr-11, in agreement with previous data and, also the previously undetected importance of Asn-4. Clearly, the first ß-turn of the a-conotoxin GI is functionally important. A similar study is being performed with a-conotoxin ImI. The result of this work will be presented and discussed in the frame of the comparative analysis mentionned above.

P-002Mo

FUNCTIONAL EFFECTS OF BgK, A STRUCTURALLY NEW K^+ CHANNEL TOXIN ON CLONED *SHAKER* Kv 1.2 CHANNELS.

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BgK is K^+ channel toxin from the Caribbean sea anemone *Bunodosoma granulifera* (1). It is a protein composed of 37 amino acids that are crosslinked by three disulfide bridges (2). BgK is structurally unrelated with scorpion toxins such as charybdotoxin (ChTx) and agitoxin 2 (AgTx2). However, despite the fact that BgK shares 35% homology with the sea anemone *Stichodactyla hellanthus* toxin (ShK), there are a number of marked structural differences located mostly in the N-terminal regions (3).

The functional site of Bgk has been investigated, using alanine-substituted mutants, by assessing their ability to displace ^{125}I-α-DTx from rat brain synaptosomes and from the *shaker* Kv 1.2 channels expressed in B82 cell line. The data showed that changing five amino acid residues, i.e. K25, F6, Y26 and to a lesser extent H13 and S23, caused a decrease in the ability of BgK to compete with ^{125}I-α-DTx for its binding site in rat brain synaptosomes. With K25 no displacement was observed up to 300 nM. Moreover, binding assays performed with cloned Kv 1.2 channels produced similar results. This finding agrees with observations on ShK where K22 critically binds to Kv 1.2 channels (4). The data also suggest that the residues F6, Y26 and perhaps H13 and S23 are also part of the active site of BgK.

The functional effects of BgK are being investigated by using its mutants on B82 cells expressing cloned *shaker* Kv 1.2 channels using whole cell patch-clamp experiments. Preliminary data showed that 50 nM of BgK reduced current amplitude by 75% of the control. However 50 nM of F6, H13, S23 and Y26 blocked the K^+ currents by 25, 50, 45 and 30%, respectively. Interestingly, K25 (50 nM) was unable to block the Kv 1.2 channels.

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We thank ORS for CLCM's scholarship and Prof. S. Grissmer for supplying B82 cells expressing cloned *shaker* Kv 1.2 channels.

P-003Mo

A STRATEGY FOR MODELING THE COMPLEX OF CN2, A SCORPION TOXIN FROM CENTRUROIDES NOXIUS HOFFMANN, WITH THE NEUTRALIZING ANTIBODY BCF2

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A monoclonal antibody, BCF2, generated against Cn2, the most abundant toxin in the venom of the scorpion *Centruroides noxius*, was shown to be able to neutralize the toxic effect of the whole venom in mice (Licea, A. et al. (1996) Toxicon 34:843-847). Inhibition studies of the antibody-antigen binding using synthetic peptides and homologous toxins showed that the conformational epitope seems to be constituted by the N-terminus in conjunction with the C-terminus, but includes an additional part of the molecule. Our analyses center especially on residues Lys8 and Arg64. We propose variants of a three-dimensional model of the complex between Cn2 and BCF2, based on the crystallographic structure of the Fab fragment of antibody Jel42 complexed with its small-protein antigen HPr. First, docking was done by manually placing the putative epitope near the antigen-binding site of BCF2 guided by the position of HPr. Various versions were generated that allow interactions via Lys8 or Arg64. As a second step a protocol using the program X-Plor was applied: 50 cycles each containing a "rigid" minimization (changing the relative position of the two molecules which were treated as rigid entities) and then an unrestrained molecular dynamics of the whole complex. The quality of the models was evaluated by the number of interactions, the size of the contact surface and the concordance with the inhibition experiments. (supported by Howard Hughes Medical Inst. 75/97-527107 and DGAPA-UNAM IN211996).

P-004Mo

COBATOXINS 1 AND 2 FROM CENTRUROIDES NOXIUS HOFFMANN REPRESENT A NEW SUBFAMILY OF K^+ CHANNEL BLOCKING TOXINS

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Short-chain neurotoxins from scorpions represent valuable tools to study structure-function-relationships of K^+ channels. Here we report two new toxins isolated from the Mexican scorpion *Centruroides noxius* Hoffmann, that were named cobatoxin 1 and 2. The primary structures were determined by direct sequencing and mass spectrometry. The ability of the cobatoxins to block K+ channels were tested by displacement experiments in membranes from rat brain synaptosomes and by electrophysiological experiments using the K channel *Shaker* B expressed in insect cells Sf9. Cobatoxin 1 and 2 displace [^{125}I]noxiustoxin from its binding sites in rat brain synaptosomes with IC50s of around 2nM. Both toxins block the *Shaker* B channel with an K_D of 0.7 μM and 4.1 μM, respectively. The cDNA of cobatoxin 1 was isolated from a cDNA library. It codes for a signal peptide of 28 amino acids and a pre-peptide of 34 amino acids. In the mature peptide the last two residues (Gly33, Lys34) are processed to leave the C-terminus Tyr32 amidated. A sequence comparison of the cobatoxins with homologous short-chain scorpion toxins revealed that they may represent a new sub-family. A three-dimensional model of cobatoxin 1 is used to analyze the spacial situation of particular residues thought to be involved in putative K-channel binding sites. (supported by Howard Hughes Medical Inst. 75/97-527107)

P-005Mo

TRANSIENT K^+-CURRENTS FROM CEREBELLUM GRANULAR CELLS ARE COMPLETELY BLOCKED BY A PEPTIDE PURIFIED FROM THE VENOM OF THE SCORPION *Androctonus australis* GARZONI

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The outward potassium currents in rat cerebellum granular cells were studied with the patch-clamp technique in the whole cell configuration system. Two voltage dependent components were identified: a) a slow activating current characterized by non-inactivating kinetics and blocked by external application of 20 mM tetraethylammonium ions, with properties similar to the classical delayed-rectifier potassium channels; b) a component characterized by fast activating and inactivating kinetics, blocked by external application of 4 mM 4-aminopyridine and similar to I_A-type currents. We have investigated the modifications of these currents by external application of 2 μM concentration of peptide Aa1 (from *Androctonus australis*, toxin 1). Peptide Aa1 was purified by chromatographic techniques (Sephadex G-50, followed by HLPC separations). This toxin was able to block the I_A-type current with an IC_{50} value around 130 nM, whereas the other non-inactivating currents were not affected by toxin Aa1, in the same experimental conditions.

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P-006Mo

Amino acid sequences, pharmacological properties, and molecular modeling of the two most potent β-neurotoxins isolated from the venom of the Mexican scorpion *Centruroides suffusus suffusus*.

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The amino acid sequences of two β-type new toxins, toxin IV and toxin VI from the venom of the Mexican scorpion *Centruroides suffusus suffusus*, have been determined by Edman automatic degradation of the entire reduced and alkylated proteins, and of peptides obtained by enzymatic cleavage.Both sequences are 66 residue long and differ from each other by only three residues. They are highly homologous with the toxin Css II from the same scorpion, but they are several times more potent. In the process of binding to rat brain sodium channels, association and dissociation kinetic constants of both toxins accounted for their very high affinity for their receptor. Both toxins were reacted with a photoreactive reagent and gave derivatives which behaved differently in their covalent association to rat brain neuronal membranes. We propose to discuss the pharmacological properties of the native toxins and their photoactivable derivatives on the basis of three-dimensional models obtained by homology modeling.

P-007Mo

Structure-activity relationship studies of the toxin II from the scorpion *Androctonus australis hector*: modification of lysine and arginine residues.

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The lysine residues the α-toxin Aah II from the venom of the scorpion *Androctonus australis hector* have been chemically modified. Acetylation of Aah II mainly generated the Lys58 monoacetylated derivative. The Lys58 acetylation dramatically lowered the pharmacological activity of Aah II. No detectable side reactions occured, as indicated by endoproteinase Lys-C peptide mapping, automoatic Edman degradation, and electrospray mass spectrometry. Circular dichroism showed that acetylation of Lys58 did not alter the overall structure of the toxin. Modification of lysines by other reagents indicated that the loss of activity of the derivatives is modulated by the chemical structure of the reagent. The bulkiness of the reagent does not seem critical, but the conservation of a positively charged group appears of great importance. Our results also suggest that the reactivity of a peculiar lysine residue is mainly controlled by its local pK, depending on its micro-environment.
Aah II was also modified by the enzyme peptidylarginine deaminase, which transforms arginine residues into citrulline. The residue Arg62 appeared to be important for the activity of Aah II. Electrostatic isopotential surfaces of the native and derivatized toxin II were compared, which suggests that electrostatic interactions are very likely involved in the very high affinity of Aah II for rat brain sodium channels.

P-008Mo

EPIDEMIOLOGY OF THE ACCIDENTS CAUSED BY *Bothrops leucurus* IN THE METROPOLITAN REGION OF BAHIA, BRAZIL (1982-1996)

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Bothropic accidents represent about 94% of the snakebite envenomation in Bahia, Brazil. The present investigation is a descriptive study about the epidemiological aspects of the ophidian accidents caused by *Bothrops leucurus* in the metropolitan area of Salvador, Bahia, Brazil, from January 1982 to April 1996. These accidents were notified to the Information Centre of Antivenom (CIAVE). This study is based on medical records of 126 patients and questionnaires filled by the author who interviewed 13 patients or the persons accompanying them during the prospective phase of study (from January, 1995 to April, 1996). The 126 accidents occurred mainly in the cities of Salvador (29.3%) and Camaçari (26.1%). However, the highest incidences were reported in the cities Itanagra (119.1/100,000 inhabitants), Mata de São João (26.8/100,000 inhabitants) and Camaçari cities (25.7/100,000 inhabitants). The accidents occurred mainly in March, May, June, September and October. Mostly of them took place in the rural areas (56,7%) and during diurnal period (60.0%). Most of the patients were adult male rural workers, 10 to 49 years old, and 65.4% of the accidents occurred during work. The accidents involved the feet (65.9%) and the hands (25.4%). Time elapsed between the snakebite and medical care, snakebite and serotherapy, and admission to the CIAVE and serotherapy was less than six hours in 84.2%, 87.0% and 82.6% of the cases, respectively. None of the cases resulted in death indicating the efficiency of the treatment with bothropic antivenom in the metropolitan area of Salvador.

P-009Mo

THREE-DIMENSIONAL CRYSTAL STRUCTURE OF MUSCARINIC TOXIN 2 FROM *Dendroaspis angusticeps* VENOM.

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Muscarinic toxins (1) are small proteins of approximately 65 residues which are present in mamba venoms and which exert a remarkable diversity of specificities for muscarinic acetylcholine receptors (2). One of them named MT2 was isolated from *Dendroaspis angusticeps* venom, purified as described previously (2) and crystallized from ammonium sulfate by vapour diffusion in hanging drops at pH 4.5.

A full data set to 1.8Å resolution was collected from a single crystal using a Mar Research imaging plate detector on a Rigaku rotating anode generator. The protein crystallizes in a trigonal unit cell with a=b=63A, c=37A $\alpha=\beta=90°$ and $\gamma=120°$ and belongs to space group $P3_221$.

The 3D structure of MT2 was solved by molecular replacement using the program AMoRe. A satisfactory solution was reached using the 3D structure of neurotoxin I from *Naja naja oxiana*, as a starting model.. The model structure was then refined by simulated annelling (T=3000°K) using XPLOR and some manual intervention, on the basis of (2 Fobs - Fcal) maps using the interactive molecular graphic TURBO program.

The structure shows that MT2 adopts a three-fingered fold, with three major loops that are connected to a more compact core where the four toxin disulfide bonds are located. This finding agrees with the NMR solution structure which was previously described (3). However, a number of differences also emerged between the two structures. These differences will be presented and discussed.

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P-010Mo

ANALYSIS OF SECRETED FLUIDS FROM *SCOLOPENDRA SUSPINIPES*

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From live animals collected in New-Caledonia we have studied secreted fluids from venom glands.

The fluids contain some toxic components which paralize insects.

The secreted fluids were mixed with 8M urea and the unfolded proteins were resolved on IEF/SDS PAGE.

Major proteins were analyzed using microsequencing and amino acids analysis.

The fluids consist of numerous isoforms of proteins which will be presented.

P-011Mo

Three dimensional structure of Calcicludine, an L-type calcium channel inhibitor extracted from the green mamba venom

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Voltage-dependant calcium channels are essential for the expression of a wide range of cellular functions including muscle contraction, neurotransmettor and hormone secretion. Five types of calcium channels (T, L, N, P/Q and R) have been described on the basis of their biophysical and pharmacological properties. Type-L channels are widely distributed in excitable cells. They are characterized by their sensitivity to dihydropyridines, which are used in the treatment of cardiovascular diseases.

Calcicludine, a sixty amino acid protein extracted from green mamba venom, specifically inhibits type-L calcium channels in cerebellar granule neurons of rat brain (Schweitz et al., 1994) and in Xenopus oocytes (Diochot et al., 1997). In order to investigate structure / function relationship in this molecule, we determined its three-dimensional structure. Calcicludine adopts the BPTI fold, formed by an α-helix and a β-sheet linked by three disulfide bridges. Therefore, it presents the same structural motif as α-dendrotoxin, another protein extracted from the green mamba venom (Sharzynski, 1992). Interestingly, while calcicludine binds to calcium channels, α-dendrotoxin binds to potassium channels. Comparison of the sequences of these two proteins, analysis of the mutagenesis data of another calcium channel blocker, ω-conotoxin GVIA (Kim et al., 1994), and knowledge of the active site of α-dendrotoxin (see poster by Gasparini et al.) led us to propose a localization fo the functional site of calcicludine.

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P-012Mo

CHARACTERIZATION OF α-NEUROTOXIN AND PHOSPHOLIPASE A_2 ACTIVITIES FROM *MICRURUS* VENOMS: Determination of amino acid sequence and receptor binding abilty of the major α-neurotoxin from *micrurus nigrocinctus nigrocinctus*

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New World elapids are coral snakes that belong to the genus *Micrurus*, and for which the venom biochemistry is mostly unknown. Analysis has been difficult because the coral snakes produce small quantities of venom. Clinical observations following bites show mainly neurotoxic effects. Experimentally, cardiotoxic, haemolytic, and myotoxic activities are also reported. An experimental approach, using reverse-phase high performance liquid chromatography and specific assays for α-neurotoxin and phospholipase A2 activities, was conducted on milligram quantities of venoms from three species from Costa Rica: *M. nigrocinctus nigrocinctus, M. alleni yatesi*, and *M. multifasciatus*. Neurotoxicity was determined by competition binding experiments with the *Torpedo marmorata* acetylcholine receptor. Phospholipase A_2 activity was measured by fluorimetry using a pyrene lipid substrate. In this way, we purified and characterized seven α-neurotoxins, five phospholipases A_2, and four toxin homologs. The amino acid sequence of the major α-neurotoxin from *M. nigrocinctus nigrocinctus* venom was fully determined and compared to Old Word representatives. Distance matrix data were generated to set up phylogeny relationships among elapid short-chain α-neurotoxins, which proved to be in accordance with taxonomic classification and geographical distribution of snake species.

P-013Mo

***Tityus caripitensis n. sp.* SCORPION VENOM GLAND HISTOLOGY**

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We have studied the histology of the venom gland of adult females of *Tityus caripitensis n. sp.* (Quiroga, 1988) (Buthidae) a scorpion responsible of severe human envenomation in eastern Venezuela. The animals were captured and kept as indicated by Quiroga *et al.* (1982). The animals were weighted and their telson removed 24h after drawing venom, glands were fixed 12h in Bouin solution. Paraffin sections were colored with either Hematoxilin Eosin or Mason staining. The gland is made of two ovoid lobes that fill the vesicle except a small cavity where the venom accumulates; the cavity continuates distally with the excretory duct. Striated muscle inserted in the cuticle surrounds the middle of the lobes. These lobes are made of a simple, pseudo stratified epithelium organized in five folds which increase in thickness toward the telson. The epithelium contains secretory cells having either coarse or thin granules, basal cells and nonsecretory cells. The diversity of the granules suggests that they may represent different secretory materials rather than to distinct stages of venom processing as suggested by some authors. The central axis of the lobes is made of very thin connective tissue with fibroblasts and cells resembling mast cells which contain eosinophilic inclusions. The excretory duct is lined by a simple cylindric ciliated epithelium supported by connective tissue rich in collagen fibers and surrounded by the chitinous epidermal tissue. The secretory cell content differs from the observed in *Centruroides* and *Diplocentrus* scorpions, but resembles *Buthus martensi* from Manchuria. **Acknowledgements:** Supported in part by FUNDACITE GUAYANA; Venezuela.

P-014Mo

ACTION OF CENTIPEDE *SCOLOPENDRA SP.* VENOM ON THE GIANT AXON AND ON THE CHOLINERGIC CENTRAL SYNAPTIC TRANSMISSION OF THE COCKROACH *PERIPLANETA AMERICANA.*

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Existence and characterization of possible neurotoxins in Centipede venoms have not been much studied. The crude venom (CV) and a fraction (SC1) isolated by gel filtration have been tested, for the first time, in current and voltage-clamp, on an insect isolated giant axon using the double oil-gap method. CV (up to 4 mg/ml) had no appreciable effect; SC1 (0.2 to 1 mg/ml) depolarized the axon (5 to 20 mV), blocked the evoked action potentials in 2 to 10 minutes, but also decreased membrane resistance. In voltage-clamp, when Na^+ and K^+ currents were both decreased (10 to 40%), their kinetics were not appreciably modified and the leak current was irreversibly increased. It is concluded that the most active fraction of the venom SC1 may open non-specific ion pores in the axonal membrane but that neurotoxins specifically acting on Na^+ or K^+ channels could not be detected in this venom. To prove effects on cholinergic central synaptic transmission, the CV (10 µl, 1 mg/ml), not active at this concentration on the axonal membrane, was directly injected into the sixth ganglion neuropile where cercal afferent - giant interneuron synapses are located. The microinjection induced (1) a small transient depolarization (1.5 mV, 10 s) of postsynaptic membrane, followed by a slow durable (12 to 15 min) depolarization wave reaching 3-4 mV, (2) a drastic decrease in the evoked subthreshold excitatory post synaptic potential amplitude. A pretreatment of the ganglion with 10 µM atropine or scopolamine reduced the amplitude of the venom induced slow depolarizing wave, suggesting a possible cholinergic muscarinic target for *Scolopendra sp.* venom.

P-015Mo

IMPLICATION OF 8-10 REGION OF AN ALPHA TYPE RECOMBINANT SCORPION TOXIN IN ITS ELECTROPHYSIOLOGICAL PROPERTIES

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Bot XIVr. is a recombinant toxin from a *Buthus occitanus tunetanus* venom gland cDNA library. This insect toxin was produced in *E. coli* as a fusion protein with two IgG binding (Z) domains derived from protein A of *Staphylococcus aureus* and was characterized as an α-type toxin that provides an efficient immune protection against structurally related mammal toxins (Bouhaouala-Zahar *et al.*, 1996). With the objective to study this toxic specificity and to determine functional sites of this toxin by genetic manipulation, the cDNA clone encoding BotXIVr has been used to produce a recombinant variant of the toxin in the same efficient bacterial expression system. The directed mutation concerns the modification of BotXIVr 8-10 sequence QPH by KNY sequence of LqhαIT. This alteration induces a non lethal reversible contractive paralysis of the insect body. Electrophysiological studies are carried on the adult male *Periplaneta americana* giant axon in double oil-gap technique. In current clamp conditions, M8-10 induces an unusual and slight membrane depolarization associated to a prolonged action potential duration resulting in a "plateau" shape as seen with the a recombinant toxin. In voltage clamp, the peak inward sodium current amplitude decreases and a dose-dependent maintained inward current develops. Sodium current inactivation and activation curves are both shifted toward more negative potentials. Thus, M8-10 affects in the same time the sodium current activation and inactivation mechanisms contrary to BotXIVr which affects only the inactivation process. This "duality" of action suggests the implication of residues 8-10 in the functional activity of the Bot XIVr.

Bouhaouala-Zahar et al. (1996), *Eur. J. Biochem.*, 238, 653-660.

P-016Mo

Lys-77 IS IMPORTANT FOR HEMOLYTIC ACTIVITY OF EQUINATOXIN II, A PORE FORMING TOXIN FROM THE SEA ANEMONE *Actinia equina*

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Equinatoxin II (EqtII) is potent lysin isolated from the sea anemone *Actinia equina*. The mechanism of action, at the molecular level, is still poorly understood, but involves the assembly of an oligomeric pore onto target cells. In order to study it, the mutant K77C was expressed and purified from *E. coli*. It' s hemolytic activity was reduced more than 100 times as compared to the wild-type. It was further diminished, if negative charge was introduced by modification of cysteine with iodoacetic acid. On the other hand, the activity on red blood cells was restored back after modification with bromoethylamine. EqtII K77C releases calcein from lipid vesicles with higher cooperativity than wild-type toxin. Sugar protection experiments, on red blood cells, showed that mutant forms larger pores. After the modification with bromoethylamine pores are smaller again. The mutation and chemical modifications showed no effect on binding to erythrocytes, since all three forms of toxin bind to red blood cells equally well. Furthermore, once the toxin is bound to the lipid vesicles, Lys77 is located on the outside of the vesicles. CD spectra of wild-type and the K77C mutant do not show significant differences in amount of different secondary structures. It appears that this residue is not involved in binding of the toxin to the membrane but it is rather important either for interaction with monomers or for the modulation of ion flow through the pore.

P-017Mo

Expression of intermediate filament proteins in mulgotoxin induced myodegeneration.

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Intermediate filaments are a main constituent of the exosarcoplasmic cytoskeleton of muscle fibres. These filaments interlink the myofibrils at the Z disk level and also connect them to the sarcolemma and the nuclear envelop. Our previous studies have shown that mulgotoxin induced disruption of sarcolemmal membrane and early selective degeneration of Z disk. Hence we decided to investigate the role of intermediate proteins desmin, titin and vimentin in mulgotoxin induced myodegeneration in experimental mice and rats, by immunohistochemistry and immunofluorescence techniques. We found that desmin, titin and vimentin were almost absent in necrotic muscle fibres by 3h, 6h, and 24h after injection of mulgotoxin. However early loss of desmin was noticed from as early as 15 min and 1h, during which titin and vimentin were still expressed. Desmin was almost absent in delta lesions, which were generally observed in early myodegeneration. Ultrastructural gold immuno labelling with anti-mulgotoxin by immunoelectron microscopy showed internalisation of mulgotoxin inside the sarcopalsmic reticulum, mainly around the delta lesions, by 1h after injection. We conclude that intermediate filament proteins are rapidly destroyed once the toxin gets internalised into the muscle fibres resulting in loss of myofibril Z disk assembly, disruption of muscle architecture and myodegeneration.

P-018Mo

CHIBUTOXIN, A NOVEL TOXIN ISOLATED FROM THE VENOM OF THE SCORPION *BUTHUS MARTENSI* KARSCH WHICH INHIBITS ADRENERGIC TRANSMISSION

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The crude venom (MKV) of the scorpion *Buthus martensi* Karsch has been shown to mediate adrenergic actions in the rat anococcygeus muscle (Acm). By gel filtration on a Bio-gel P-30 Column, MKV was fractionated into seven fractions, MK1 to MK7, of which only MK2 showed inhibitory adrenergic effects. A novel toxin (Chibutoxin, CbTx) which can inhibit adrenergic responses was isolated from MK2 by further gel filtration on Bio-gel P-10 column. The purity of CbTx was assessed by capillary electrophoresis and found to be homogeneous. The effects of CbTx on adrenergic responses were investigated using the rat isolated Acm mounted in Krebs solution (37°C, 5% CO_2 in O_2). CbTx (10 μg/ml) markedly inhibited the contractions (adrenergic responses) induced by field stimulation (20-30V, 10 Hz for 10 sec, 1 msec pulse width, every 1 min), but not the responses to 3 μM noradrenaline (NA), 3 μM carbachol (CCh) and 50 mM KCl. Interestingly, the contractile responses of 4 μg/ml Makatoxin II (MkTx II) from the same venom and MKV itself (10 μg/ml) were also blocked by CbTx. CbTx is, therefore, a novel toxin which can inhibit adrenergic responses through some prejunctional mechanism(s) of action in the rat Acm.

P-019Mo

CAPILLARY ELECTROPHORESTIC ANALYSIS OF SCORPION VENOMS

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In order to obtain a basic understanding of the electrophoretic properties of scorpion venoms and to determine whether or not the electrophoretic patterns are specific for morphologically distinct species, capillary electrophoresis were conducted on scorpion venoms from different species representing *Androctonus, Buthus, Buthotus, Heterometrus, Leiurus*, and *Parabuthus*. The sample (1 mg/ml) was injected into the capillary (50 cm × 50 μm, uncoated) by 5 psi*sec. The CZE (capillary zone electrophoresis) was performed in 0.1M phosphate buffer under 12.00 kV voltage with current limit of 50 μA under the polarity from positive to negative for 40 min. The detection was measured at 200 nm wavelength and the electropherograms were analyzed by the BioFocus 3000 software. The profiles of venoms from different scorpion species indicate that some proteins and polypeptides components of these venoms have common electrophoretic characteristics suggesting a genetic relationship. Conversely, the electropherograms also showed the characteristic protein and polypeptide profiles that could differentiate one scorpion species from another. Therefore, the electropherogram profiles suggest that proteins and polypeptides with similar characteristics abound among species, although each venom has a unique profile that differentiates one species from the other.

P-020Mo

POLYAMINE SPIDER TOXINS AS A BASE FOR BIOSPECIFIC CHROMATOGRAPHY OF INSECT MUSCLE IONOTROPIC GLUTAMATE RECEPTOR.

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Polyamine toxins (argiopins or argiotoxins) from *Argiope lobata* spider venom are potent blockers of ionotropic glutamate receptor channels from the vertebrate central nervous and insect motor systems. They are widely used to study the mechanism of ionotropic receptor functioning. L-Quisqualate sensitive ionotropic glutamate receptors are present on the membrane of locust skeletal muscle. Present work deals with isolation of locust muscle glutamate receptors by means of biospecific chromatography on immobilized argiopins. The purified receptor complex was shown to be a glutamate receptor of quisqualate type according to electrophysiological and radioligand binding assay data. After reconstitution of complex into liposomes radioligand binding assays revealed in it binding sites for [^{3}H]-L-Glu (K_d of 0.47mkM, B_{max} of 2.3nmol/mg) and for [^{3}H]-L-Asp ((K_d of 0.65mkM, B_{max} of 1.87 nmol/mg). The binding of L-glutamate was displaced by L-quisqualate, L-aspartate, but not by kainate, AMPA and NMDA. By electrophysiological analysis of reconstituted receptor complex glutamate- and quisqualate-activated cation-selective ion channels were discovered. The receptor complex contains protein components with molecular masses 97, 94, 60 and 45 kDa. Anti-idiotypic antibodies against glutamate which could recognize insect quisqualate receptor strongly interacted with the 97 and 94 kDa bands. Structural analysis of these glutamate-quisqualate-binding subunits fragments did not indicate essential homology with other glutamate receptors. These data were not surprising because pharmacological properties of insect quisqualate receptor differ greatly from all known mammalian non-NMDA glutamate receptors.

P-021Mo

EFFECTS OF PAX ISOLATED FROM THE CORAL *PARAZOANTHUS AXINELLAE* ON NEUROMUSCULAR JUNCTION AND ON NEURONAL AChE

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It has been shown that pseudozoanthoxantins purified from the coral *Parazoanthus axinellae adriaticus* inhibit acetylcholinesterase (AChE). The inhibitory activity of the most abundant peak, named PAX, is comparable to the AChE potency of physostigmine, a classical inhibitor used in medicine [1]. Since the toxicity of PAX is very low [1] it might be useful as a starting substance for the development of new drugs for treatment of several conditions, including myasthenia gravis and Alzheimer's disease. The AChE inhibitory potency of PAX was assessed using the standard two-microelectrode voltage and current clamp technique in neuromuscular junction, and by measurements of the rate of acetylcholine hydrolysis in neuronal tissue after the application of PAX. End-plate potentials remained unchanged when using PAX in 10^{-7}M or lower concentrations. The half-decay time was doubled in 4.5 x 10^{-5}M concentration. There was also no significant change in the frequency of the miniature end-plate potentials. AChE inhibitory activity of PAX in neurons was measured using Ellman's method for the purified enzyme, and by continuous pH-metric titration at constant substrate concentrations at pH 7.4 for the membrane bound cholinesterases. The membrane-bound AChE inhibition by PAX was monitored in slices of rat brain and in bovine superior cervical ganglion. K_i in neurons was 0.78 x 10^{-6} M (brain slice) and 1.05 x 10^{-6} M (superior cervical ganglion). The results allow the conclusion that PAX preferentially inhibits neuronal AChE, which might be explained by an easier access of the substance to the enzyme in our experimental conditions.

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P-022Mo

EFFECTS OF A PHOSPHOLIPASE A_2 FRACTION FROM *B. insularis* VENOM ON THE MEMBRANE RESTING POTENTIAL AND Ca^{2+} SENSITIVITY OF FAST AND SLOW MUSCLE FIBERS

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In this work, we have examined the electrophysiological actions of a phospholipase A_2 fraction ($fPLA_2$) obtained from *B. insularis* snake venom by gel-filtration on Sephadex G-150 followed by ion-exchange chromatography. The effects of this fraction on the resting potential and contractile properties of slow (soleus, SOL) and fast (extensor digitorum longus, EDL) muscle from rats was determined by incubating the muscles in Tyrode solution containing $fPLA_2$ (40 µg/ml) for 10 min. At the end of this incubation, the solution was exchanged for one without $fPLA_2$ and the desired electrophysiological parameters then determined. Control preparations were incubated with Tyrode solution alone throughout. The $fPLA_2$ reduced the resting potential of EDL muscle by 16% ($P<0.05$, $n=3$) compared to control preparations but had no effect on that of SOL muscle ($n=3$). Skinned fibers from both types of muscle were incubated with varying concentrations of Ca^{2+} (5.0-7.0 mM) before or after incubation with $fPLA_2$ (40 µg/ml) and the resulting tension recorded via an isometric transducer. Following exposure to $fPLA_2$, the Ca^{2+}-tension curve for EDL muscle was significantly more sensitive to Ca^{2+} than that of non-treated muscle ($p<0.05$, $n=6$ for treated and non-treated fibers). In contrast, in SOL muscle, there was a significant decrease ($p<0.05$, $n=9$) in the sensitivity to Ca^{2+}, and hence in the Ca^{2+}-tension curve compared to control fibers ($n=12$). Patch clamp analysis of single muscle fibers showed that the $fPLA_2$ altered the kinetics of potassium channel currents. We conclude that the $fPLA_2$ acts by different mechanisms in the two types of muscle studied.

P-023Mo

Purification and characterization of rat stomach contractile peptide from the venom of *Agkistrodon halys blomhoffii*

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A rat stomach fundus contractile peptide was purified from *A. halys blomhoffii* venom via three steps of column chromatogrphy on Sephadex G-100, preparative reversed phase-high performance liquid chromatography(RP-HPLC) and the final RP-HPLC. This peptide, named blomhotin proved to show ephemeral contractile response to the rat stomach fundus strip. Blomhotin(BH) was consisted of 11 amino acid residues with a blocked N-terminus of pyroglutamic acid. The molecular weight was approximately 1153.6 from FAB mass spectrometry data. The amino acid sequence proposed for BH is pGlu-Gly-Arg-Pro-Pro-Gly-Pro-Pro-Ile-Pro-Arg. This peptide had been synthesized, and the synthetic peptide was found to be identical with the native blomhotin in its rat stomach fundus contractile activity and the chemical properties. The primary structure of BH was almost identical to bradykinin-potentiating peptides(BPPs) with the exception of the additional C-terminus arginine. Despite the surprisingly close structure -relationship to BPPs, inhibitory activity for angiotensin-converting enzyme was found to be extremely low in the synthetic blomhotin. In order to study structure-function relationship of BH, some BH derivatives(BH_{4-11}, BH_{1-10}, 11-Pro-BH) were chemically synthesized and their contractile activities were examined. BH_{4-11} elicited more or less the same contractile activity as BH, and BH_{1-10} and 11-Pro-BH have completely lost the activity, indicating that the C-terminal region including C-terminus arginine is responsible for the contractile activity.

P-024Mo

OCCURRENCE OF PARALYTIC SHELLFISH POISON IN THE STARFISH ASTERIAS AMURENSIS IN HIROSHIMA BAY, HIROSHIMA PREFECTURE, JAPAN

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The starfish Asterias amurensis collected in Hiroshima Bay was found to contain toxins which showed strong paralytic action in mice; the maximum toxicity (as paralytic shellfish poison, PSP) was 8.0 MU/g for whole body and 28.7 MU/g for viscera from spring to early summer in 1996 and 1997. It appeared that the toxicity was almost exclusively present in the viscera. They were extracted with 80% ethanol acidified with acetic acid, followed by defatting with dichloromethane. The aqueous layer obtained was treated with activated charcoal and then applied to a Sep-Pak C18 cartridge. The unbound toxic fraction was analyzed by HPLC. The starfish toxin was rather unexpectedly identified as PSP. It was comprised of gonyautoxins and saxitoxin group as the major components, along with protogonyautoxins, which are N-sulfocarbamoyl derivatives. It was concluded that the toxin of starfish collected in Hiroshima Bay consisted of PSP, which supposedly came from toxic bivalves living in the same area by the way of food chain. To our knowledge, this is the first report of the occurrence of PSP in starfish.

P-025Mo

DETERMINATION OF THE CLEAVAGE SITE OF THE BAIT REGION OF HUMAN α2-MACROGLOBULIN AND RAT MURINOGLOBULIN BY A HEMORRHAGIC METALLOPROTEINASE FROM *BOTHROPS JARARACA* VENOM

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Jararafibrase I (JF I) is a hemorrhagic metalloproteinase obtained from *B. jararaca* venom. We found that the purified enzyme formed complexes with murinoglobulin (MG) and α1-macroglobulin in rat serum, and α2-macroglobulin (α2-M) in human serum. We also observed that JF I cleaved the bait region of these three α-macroglobulins. In the present study, the cleavage site of rat MG and human α2-M was determined by amino acid sequence analysis. After incubation of JF I with MG or α2-M, each sample was subjected to SDS-PAGE under reducing conditions. The products separated by SDS-PAGE were electroblotted onto a PVDF membrane. NH_2-terminal-sequence analysis was then performed on the 210 kDa and 150 kDa products of MG, and the 90 kDa and 95 kDa products of α2-M using a 492-protein sequence analyzer. The cleavage site of MG was ^{686}Ala- ^{687}Val, and that of α2-M was ^{696}Arg-^{697}Leu. We reported previously that JF I cleaves the position of ^{143}Glu- ^{144}Leu in single-chain urokinase-type plasminogen activator. Based on these results, it is suggested that JF I may preferentially cleave the N-terminal of the hydrophobic group of the amino acids, valine and leucine.

P-026Mo

NEURONAL ACCEPTOR FOR AMMODYTOXIN IN PIG BRAIN CORTEX

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Ammodytoxins (Atx) are presynaptically neurotoxic phospholipases A_2 isolated from the venom of long - nosed viper (*Vipera ammodytes ammodytes*). Using radioiodinated AtxC (^{125}I-AtxC), specific binding sites were found in presynaptic membranes isolated from pig brain cortex. The binding parameters of ^{125}I-AtxC high-affinity membrane interaction are K_d: 15 nM and B_{max}: 1.5 pmol/mg of membrane protein. ^{125}I-AtxC was covalently cross-linked to its acceptor in synaptic membranes using the bifunctional reagent DSS. Subsequent SDS-PAGE analysis under reducing conditions followed by autoradiography revealed a specific adduct of apparent M_W 39 kDa. Taking in account that the stoichiometry of binding is one molecule of toxin (14 kDa) to one molecule of acceptor, the M_W of AtxC neuronal acceptor is 25 kDa. Specific binding and cross-linking of ^{125}I-AtxC to the neuronal acceptor was strongly inhibited only by AtxA. Partial inhibition was obtained by agkistrodotoxin while crotoxin (*Crotalus durissus terrificus*), ß-bungarotoxin (*Bungarus multicinctus*), OS_2 and taipoxin (*Oxyuranus scutellatus*), all ß-neurotoxic phospholipases A_2, showed no inhibition. Ammodytin L, a phospholipase A_2 analogue with myotoxic activity, and ammodytin I_2, a non-toxic phospholipase A_2, both from *Vipera ammodytes ammodytes* venom, were also not able to displace ^{125}I-AtxC from its specific binding site on the presynaptic membrane. AtxC neuronal acceptor is protein, since proteinase and heat treated membranes lost the toxin-binding ability.

P-027Mo

PROTEIN ENGINEERING OF THE C-TERMINAL HALF OF AMMODYTOXIN A

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Ammodytoxin A (AtxA) is the most presynaptically neurotoxic phospholipase A_2 (PLA_2) from the *Vipera a. ammodytes* venom. Its mode of action has not yet been completely elucidated. In order to find amino acid residues in the molecule responsible for neurotoxicity, including those participating in the primary binding to a specific neuronal acceptor, we approached this problem by protein engineering. A cDNA encoding wild-type atxA was inserted into a T7 RNA polymerase-based expression vector and recombinant toxin produced in inactive form as inclusion bodies in the cytoplasm of *Escherichia coli*. The inclusion bodies were isolated, recombinant ammodytoxin renatured by an *in vitro* procedure and properly folded toxin purified by cation-exchange chromatography. Several mutants were prepared by site-directed mutagenesis and polymerase chain reaction, produced in the bacterial expression system and characterized. Exchange of two basic, lysine residues at positions 98 and 111 for asparagines led to an increased enzymatic activity, whereas lethality and specific binding to a neuronal acceptor were not diminished. The atxA(K128E) mutant showed no influence on PLA_2 activity and lethality, but, surprisingly, it has slightly lower affinity for the membrane acceptor. This finding suggests that a single amino acid replacement at position 124, Phe for Ile (one hydophobic residue for the other), should be responsible for about 20-fold lower toxicity of atxC from atxA. The triple mutant atxA(K65S/H67S/R68L) with a replacement of three basic residues in the β-structure had a lower lethality, higher enzymatic activity and diminished binding affinity for a low-affinity binding site.

P-028Mo

CHARACTERISATION OF MUSCARINIC TOXINS MT1, MT2, MT3 AND MT7.

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A family of muscarinic toxins (MT1, MT2, MT3 and MT7) has been isolated from the venom of the Eastern green mamba (*Dendroaspis angusticeps*). Binding studies with cloned muscarinic receptors have shown that these toxins are highly selective for individual muscarinic receptor subtypes. Ki values for MT1 are 48nM and 72nM at M_1 and M_4 receptors respectively and MT2 has Ki values of 364nM and 1200nM at M_1 and M_4 receptors respectively. MT1 and MT2 have been shown to act as agonists at the M_1 receptor subtype (1). MT3 is the most selective muscarinic ligand known for the M_4 receptor subtype (Ki = 2nM) (2) and MT7 is highly selective for the M_1 receptor subtype (Ki = 2pM) (3). It is hoped that these toxins may prove to be useful selective pharmacological tools which can be used to identify the physiological roles of the muscarinic receptor subtypes. In the rabbit vas deferens (M_1/M_4), we have shown that MT1 and MT2 cause a decrease in the twitch response to electrical stimulation, implying agonist activity at presynaptic, inhibitory muscarinic receptors. These responses to the toxins are not blocked by pirenzepine (100nM) or himbacine (100nM). The responses to the toxins are not affected by the M_1-selective allosteric modulator brucine (100μM). MT3 (50nM) and MT7 (20nM) failed to block the change in twitch responses to MT1 (100nM), MT2 (100nM), McNeil-A-343 (1μM) and carbachol (10μM). Our results demonstrate that M_1 receptor subtypes modulate transmission in this tissue. However, MT1 and MT2 appear to be interacting with an atypical M_1 receptor subtype to cause their inhibitory effects. In the guinea pig uterine preparation (M_2/M_4), MT1, MT2 and MT3 had no effect; hence the muscarinic receptor subtype in this preparation is more likely to be M_2 rather than M_4. In the guinea pig ileum longitudinal muscle preparation (M_3), neither MT1 (200nM) nor MT3 (50nM) had any effect as agonists or antagonists. MT2 produced 4-DAMP-sensitive (25nM to 1200nM) concentration-dependent contractures. The maximum obtainable contracture to MT2 was 25% of the maximum contracture by methacholine.

1. Jerusalinsky, D., Harvey, A. L. (1994) *TiPS* **15**, 424-430.
2. Jolkkonen M. et al., (1994) *Febs Letters* **352**, 91-94.
3. Jolkkonen, M. (1996) Ph.D. Thesis, University of Uppsala.

We thank Dr E. Karlsson for the gift of MT7.

P-029Mo

AUSTRALIAN FUNNEL-WEB SPIDER TOXINS AND α-SCORPION TOXINS SHARE A COMMON BINDING SITE ON COCKROACH AND RAT VOLTAGE-GATED SODIUM CHANNELS

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Versutoxin (VTX) and robustoxin (RTX) are lethal peptide neurotoxins isolated from the venom of Australian funnel-web spiders. Previous studies using whole-cell patch clamp recording in rat dorsal root ganglion neurons have shown that these toxins interact with the voltage-gated sodium channel to slow or remove channel inactivation (Nicholson *et al.*, 1994). To further identify the site of action of these toxins we have investigated their effects on $^{22}Na^+$ flux and radiolabelled neurotoxin binding assays. We have shown that VTX and RTX directly activate $^{22}Na^+$ flux in rat brain synaptosomes, whilst partially inhibiting the flux activated by the full agonist batrachotoxin (BTX). In addition, VTX converts the partial agonist veratridine to a full agonist and partially enhances $^{22}Na^+$ flux activated by low concentrations of BTX. The observed inhibition of BTX-activated $^{22}Na^+$ flux is not mediated through an interaction with neurotoxin receptor site 1 of the channel since high concentrations of VTX failed to displace [^{3}H]-saxitoxin from this binding site. Competition binding experiments performed using [^{3}H]-batrachotoxinin A 20-α-benzoate showed that VTX causes a voltage-independent, 5-fold enhancement of binding, an action comparable to that of α-scorpion toxins. Moreover, the funnel-web spider toxins were found to potently inhibit the binding of the insect and mammalian selective α-scorpion toxins LqhαIT and AaHII to cockroach neuronal membranes and whole rat brain synaptosomes respectively. The IC_{50} values for inhibition of ^{125}I-LqhαIT binding by RTX and VTX were 0.36 nM and 0.41 nM respectively, whilst those for ^{125}I-AaHII binding were 1 nM for RTX and 6 nM for VTX. These studies indicate that the site and mode of action of Australian funnel-web spider toxins is similar to α-scorpion toxins which bind to neurotoxin receptor site 3 on the voltage-gated sodium channel.

Nicholson G.M., Willow M., Howden M.E.H. and Narahashi T. (1994) *Pflügers Arch. (Eur. J. Physiol.)*, 428, 400-409.

P-030Mo

MODIFICATION OF STICHOLYSIN II BY FREE RADICALS

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Sticholysin II (St II) is a basic 20 kDa polypeptide purified from the sea anemone *Stichodactyla helianthus*. St II exhibits potent hemolytic activity and increases membrane permeability by forming oligomeric pores. St II has been sequenced showing 11 tyrosine and 5 tryptophane (Trp) residues. These aromatic amino acids are potential targets for the action of free radicals. In the present work the effect of 2,2´-azobis(2-amindinepropane), ABAP: a thermal-generator of free radicals, on St II activity and fluorescence properties has been studied. Incubation of St II with ABAP at 45°C produced a significant decrease in the hemolytic activity of the polypeptide and a decay in the intrinsic fluorescence of St II Trp. The kinetics of hemolysis induced by free radical-modified St II was compared with the non modified polypeptide and allowed to estimate the proportion of non modified St II at different incubation times. Incorporation of the cytolysin into phosphatidylcholine:sphingomyelin vesicles partially protected St II from free radical damage, in contrast with St II in solution. Analysis of amino acid composition of modified St II revealed a deacrese in Trp content in agreement with the loss of intrinsic fluorescence. Free radicals dramatically affected the hemolytic activity of St II demonstrating a loss of the lytically competent conformation of the protein caused, at least partially, by damage in Trp residues. Trp in St II are extremely reactive to free radicals suggesting a high degree of exposition to the aqueous environment, nevertheless, when St II is associated to liposomes part of the Trp residues become incorporated into the bilayer resulting protected from the action of free radicals.

P-031Mo

Anti parasites effects of *Bothrops jararaca* venom

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1-LBCT-CBB- UENF , Campos , RJ, Brasil 2. DUBC, FIOCRUZ, , RJ, Brasil. 3. LQFPP-CBB-UENF, Campos , RJ, Brasil 4-DBM-IBC-UFRJ-RJ 5- IBCCF-UFRJ-RJ

Bothrops jararaca is a south-American snake of the great incidence nationally and easy maintenance in captivity . Our biological models were *Trypanosoma cruzi* and *Leishmaia major*, which are digenetic parasites, that still infect million of people in South America. We had shown previously that the venom of *Bothrops jararaca* inhibits the growth of *Trypanosoma cruzi* and *Leishmaia major* (1). Here we studied the effects of isolated components of the snake venom on infective and non infective forms of parasites. The parasites were treated with different concentrations of crude venom as well as with different fractions obtained by gel filtration chromatography in Sephacryl S-100 and DEAE ionic exchange in HPLC. The only fraction in which we found inhibitory activity, that is heat sensitive, was subjected to SDS-PAGE analysis . The electrophoretic profile showed three bands of apparent mol. mass of 14, 18 and 20 kDa. The treated parasites with the semi purified fraction were kept for 7 days and then fixed with 2,5% glutaldehyde in 0.1M phosphate buffer, post-fixed with 1% osmium tetroxide, dehydrated in graded acetone, embedded in epon and analyzed in transmission electron microscope. Our results show that the effect is dose-dependent with a clear mitochondrial swelling. In order to characterize this mitochondrial effect we test a stronger respiratory chain inhibitor Na_3N. Our observations showed that the mitochondrial effect promoted by NaN_3 is similar to the venom effect. Our results suggest that the plasma membrane is permeable to the active component of the venom , or it acts in a 2°messenger pathway enclosing a great mitochondrial swelling and fragmentation of inner mitochondrial membrane. The blockage of respiratory chain could be a result of the influx of water in the organelle. We now are performing the last steps of purification of this and NH2 terminal amino acid determination.

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P-032Mo

Hemolytic and caseinolytic activities of *Megalopyge sp* (Lepidoptera-Megalopygiidae) bristles extract and cross- reactivity with antilonomic serum (Lepidoptera-Saturniidae). Rocha Campos A.C.M.[1], Andrade R.M.G.[1], Spencer P.J.[2], Geldmacher, C.[1] and Dias da Silva W[1]. Laboratório de Imunoquímica, Instituto Butantan[1]; Coordenadoria de Bioengenharia, Instituto de Pesquisas Energéticas e Nucleares[2]; São Paulo, Brazil

Caterpillars of *Megalopyge sp* are abundant in Brazil, especially during summer, when most larvae hatch and most accidents occur. One hundred cases of Saturniidae and Megalopygiidae envenoming were registered, in 1996 (Dr. Fan Hui, H.V.B., personal communication), only in Hospital Vital Brazil, São Paulo. These accidents are usually characterized by burning sensation, followed by intense local pain. The victims usually develop regional limphadenopathy, headache and a simple inflamatory dermitis constituted of erythem, edema and itching (Pesce, H. and Delgado, A. *in* Ven.Invert. vol.III, 1971)

In order to study some biological activities of this venom, we prepared a *Megalopyge* bristles extract (Mebe) and samples were assayed for hemolytic and caseinolytic activities. The extract showed a dose-dependent activity on sheep erytrocytes, independent of lecithin and calcium. Three hundred microliters of Mebe (2mg/ml) caused 70,42% of erytrocytic lysis, while fifty microliters of Mebe were capable of causing a lysis representative of 13,79% , as compared to complete lysis caused by water. The caseinolytic activity, assayed by Kunitz, modified by Lomonte, was 53 U/mg. Components of Mebe were recognized by horse and rabbit antilonomic sera by ELISA. The chromatographic profile of Mebe, obtained by the TSK G3000 SW column in a HPLC system, presented 10 well defined and reproducible proteic peaks from 10 to 54,2 Kda, that, by SDS-PAGE and immunoblotting, didn't react with a conserved phospholipase active site antibody. We concluded that. 1) *Megalopyge* bristles extract (Mebe) can be useful (a good tool) to study Megalopygidae venom activities 2) Mebe is constituted of a pool of proteins, most of them of low molecular weigth; 3) at least one of these proteins is the responsible for the specific activity of lysing directly blood erytrocytes; 4) the *Megalopyge* bristles extract presents a relatively high proteolytic activity, 5) its protein repertoire shares some homologies with some epitopes present in *Lonomia obliqua* extracts (Lepidoptera-Saturniidae), although they belong to two different families (Saturnidae and Megalopygidae). Moreover, the results suggest that Mebe protein repertoire doesn't include any phospholipase-like protein, and the cross-reactivity with antilonomic serum should be due to other homologous antigens than phospholipases present in *Lonomia* venom. *Supported by CNPq, FAPESP, Fundação Butantan*

P-033Mo

LONOMIATOXIN: A 20 kDa PROTEIN PRESENT IN BRISTLES EXTRACTS OF *Lonomia obliqua* CATERPILLARS EXTRACTS

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1-L Imunoquímica I. Butantã, 2-DBM-ICB-UFRJ-RJ, 3-LQFFP-CBB-UENF, 4-LBR-CBB-UENF

Lonomia obliqua caterpillars*(Lepidoptra, Saturnidae)* occurs in Venezuela, French Guyana and Brazil. Envenomation resulting from the contact of the insect bristles with the skin of the victims have been reported since 1989, and in Brazil most of them occurred in Passo Fundo, State of Rio Grande do Sul and in Chapecó,State of Santa Catarina, located in the South part of the country, mainly during the rain period covering the months of October to March. Recent reports are indicating the a spread of the caterpillars is happening toward the States of Paraná and São Paulo.

The envenomation symptoms and signals are: local pain, erithema, edema, regional burning sensation and lymph nodes swelling, followed or coincident with hemoglobinuria, hematuria and sometimes death. This report describes the isolation and molecular characterization of a *Lonomia* toxin, tentatively designated as lonomina. This protein has been isolated by different steps including gel filtration, and reverse phase chromotografies. The purified 20 kDa protein has a N-terminal amino acid sequence (D-V-V-I-D-G-A-S-P-S-M-K-A-V-S-K-S-D-M-N-A-Y-Q-G) homologous to a hemolytic glycoprotein toxin from the sea anemone *Actinia equina* and it is recognized both by horse and rabbit antisera raised against crude brites of *L. obliqua* extracts in ELISA and immunoblotting assays. The protein possess a phospholipase A2 (PLA-2)-like activity as determined by its ability to induces hemolysis of sheep red blood cells in the presence of lecithin as isolecithin source. Since PLA-2 can be mediator of various biological phenomena including besides hemolysis, liberation of araccdonic acid from the phospholipides-cell membrane which is an important source of inflammatory mediators, indirect action on blood clot system and cell signalization, it may contribute to the pathogenesis of the lesions and symptoms observed during the envenomation by
L. obliqua caterpillars.

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P-034Mo

Metabolite profile of marine derived fungi.

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The present collection of fungi originate from the marine environment. Thus at least some presumably are marine species or adapted to life in the sea. Terrestrial fungi sometimes exhibit variation in the secondary metabolite profiles with changes in the culture medium. Accordingly it would be interesting to know whether the metabolites expressed in these isolates are correlated with the special environment from which they originate. The crucial difference between the terrestrial and the marine environment is the salinity of the sea. In this presentation results of growing marine derived fungi on the same medium prepared with either sea water (contains 3.5 % sea salt) or fresh water will be presented. The metabolite HPLC profile of ($EtOAc:CHCl_3:MeOH$ 3:2:1) extracts are compared by the UV max plot. The antibacterial activities are measured by testing against two human pathogenic bacteria, the Gram-positive organism *Staphylococcus aureus* and the marine Gram-negative species *Vibrio parahaemolyticus*.

P-035Mo

PURIFICACION AND PARTIAL CHARACTERIZATION OF A PROTEINASE INHIBITOR FROM SEA ANEMONE *CONDYLACTIS GIGANTEA*.

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A novel proteinase inhibitor was isolated from the sea anemone *C.gigantea* by three purification steps: trichloroacetic acid treatment of the aqueous extract, affinity chromatography on trypsin-Sepharose and gel filtracion. The molecular mass of the major inhibitor (CgPI-1) obtained by gel filtration was approximately 5000-6000 Da. The partial aminoacid sequence was determined by automatic sequencing. This sequence was compared with those described for proteinase inhibitors isolated from *Stichodactyla heliantus* (ShPI-1, ShPI-2) and sequences reported in SwissProt-database for other proteinase inhibitors. Similarity to inhibitors belonging to Kunitz family was observed. CgPI-1 exhibits activity only againts serine proteinase, such as trypsin and chymotrypsin. Ki values were determined.

P-036Mo

PURIFICATION AND PARTIAL CHARACTERIZATION OF A NOVEL PROTEINASE INHIBITOR FROM THE SEA ANEMONE *STICHODACTYLA HELIANTHUS*.

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Isolation of proteinase inhibitors from the sea anemone *Stichodactyla helianthus* was achieved by trichloroacetic acid treatment of the aqueous extract followed by affinity chromatography on trypsin-Sepharose and ion-exchange chromatography on CM-cellulose. The chromatographic profile of the latter reveals two active fractions against trypsin. The first one corresponds to ShPI-1 and the second is heterogenous. Gel filtration or reversed phase HPLC of this fraction allows the purification to homogeneity of a novel proteinase inhibitor (ShPI-2). The average molecular mass of the inhibitor obtained by fast atom bombardment mass spectrometry (FAB-MS) was 6195 Da. The aminoacid sequence was determined by automatic sequencing. The sequence of ShPI-2 (55 aminoacids) was compared with ShPI-1 and those reported in the SwissProt database for several proteinase inhibitors. Significant similarity to ShPI-1 (92 %) and proteinase inhibitors belonging to the Kunitz family was observed. ShPI-2 was active against trypsin and chymotrypsin. The dissociation constants of the complexes formed with these enzymes were determined.

P-037Mo

ISOLATION OF TOXINS FROM THE CARIBBEAN SEA ANEMONES *Bunodosoma granulifera* and *Phyllactis flosculifera.*

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BgK, the first toxin affecting potassium channels isolated from a marine organism, was purified from the exudate of the sea anemone *Bunodosoma granulifera*, and it represents a new structural type of potassium channel peptide toxin (Anciros et al., 1993). Another compound, a purine derivative (Bain) isolated also from *Bunodosoma* secretions by Sephadex G-50 gel filtration and HPLC on a C18 reversed phase column, increased I_{CaL} (0.5-1 mg/ml) and I_{K1} (1-10 mg/ml) on ventricular mammalian cardiomyocytes (Salinas et al., 1997). On the other hand, by a combination of gel filtration and ion exchange chromatography, five toxic basic polypeptides of M.W. of 4000-7000 have been isolated from a whole body ethanol extract of *Bunodosoma granulifera.* The toxicity of these peptides have been tested on the shore crab *Carcinus maenas* and on the pharmacological profile of albino mice, where they produce symptoms indicating that this compounds produce an important modification of the central nervous system activity. *Phyllactis flosculifera* is another common sea anemone. A glutamate receptor antagonist in snail and amphibian neurons, has been detected in a chromatographic fraction of the exudate of this anemone (Garateix et al., 1996). Recently, five peptide toxins were isolated from whole body ethanol extract of this animal by gel filtration on Sephadex G-50, ion exchange chromatography on SP Sephadex C-25, and chromatography on the polystirene resin Serdolit AD2. They were all toxic to crabs. The characterization of these compounds is subject of research now.

Anciros et al. (1993) Biochimica et Biophysica Acta, 1157; 86-92.
Garateix et al. (1996) Toxicon, 34; 443-450.
Salinas et al. (1997) Toxicon, in press.

P-038Mo

AMINO ACID SEQUENCE OF THE TOXIC SPIDER PEPTIDE CSTX-2 AND ITS STRUCTURAL AND PHYSIOLOGICAL DIFFERENCES TO CSTX-1

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CSTX-1 is the main toxic peptide in the venom *of Cupiennius salei* and occurs in an average concentration of 27.3 µg/µl crude venom. CSTX-2 has a concentration of 0.9 µg/µl crude venom, i.e. only one thirtieth. The lethal effect of CSTX-2 (LD_{50} for *Protophormia* 209.2 ng/mg) is 20 times weaker than of CSTX-1 (LD_{50} for *Protophormia* 10.5 ng/mg). CSTX-2 has a molecular mass of 6865.8 Da determined by electrospray MS and contains 61 amino acid which are in all positions identically with CSTX-1 (molecular mass 8352.6 Da containing 74 amino acids). The only difference to CSTX-1 is the absent of the last 13 amino acids. This C-terminal rest, therefore, seems to be responsible for the neurotoxic difference which we have observed between CSTX-1 and CSTX-2.

The last 13 amino acids of CSTX-1 were synthesized by Schafer-N Copenhagen, Denmark. This synthetic oligopeptide was controlled by HPLC and electrospray MS and has a molecular mass of 1504,81 Da. This value exactly matches the 13 amino acid difference between CSTX-1 and CSTX-2. When we first tested this oligopeptide in a *Protophormia* bioassay we found only light paralytic effects at high concentrations (2,1 µg oligopeptide/mg fly). Later we used additionally a *Drosophila* biotest which was found to be more sensitive. The effective dose ED_{50} was 0,242 µg oligopeptide/mg fly and the lethal dose LD_{50} was 0.895 µg/mg fly. This means that the synthetic oligopeptide is much less toxic than CSTX-1 or CSTX-2. However, since it was unexpected that such a small peptide shows remarkable toxic properties, we assume that it contributes to the increase of toxicity from CSTX-2 to CSTX-1.

P-039Mo

CSTX-4, A NOVEL BACTERICIDAL AND INSECTICIDAL PEPTIDE IN THE VENOM OF *CUPIENNIUS SALEI*

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Several peptides with insecticidal properties (3 - 8.5 kDa) could already be characterized in the venom of the ctenid spider *Cupiennius salei*. Now we isolated by HPLC a group of additional peptides in the molecular size range from 3 to 3.7 kDa which shows insecticidal and also bactericidal effects in different biotests. One very active peptide was purified and its amino acid sequence of a total of 34 amino acids was determined until position 24. The molecular mass is 3701.25 Da and was measured by electrospray MS. The peptide possesses no histidine, arginine and cysteine, but shows a high content of alanine, leucine (5 mol/mol peptid) and lysine (7 mol/mol peptide).

In our *Drosophila* bioassay a test volume of 0.05 µl can be injected near coxa I of anesthetized flies. The effective dose ED_{50} of CSTX-4 is 10,3 ng/mg fly and the lethal dose LD_{50} is 66.4 ng/mg fly, so it belongs to the most potent toxins in the venom of *Cupiennius salei*. In an agar diffusion test with *Bacillus subtilis* the peptide shows growth inhibition in a concentration of 830 µM.

P-040Mo

Bothrops jararaca SNAKES PRODUCE SEVERAL BOTHROJARACIN ISOFORMS FOLLOWING AN INDIVIDUAL PATTERN

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More than one isoform of bothrojaracin (BJC), a potent and specific thrombin inhibitor isolated from *Bothrops jararaca* venom, has been found in individual venoms collected from adult snakes. Variations in snake venom composition have previously been associated with factors such as age, sex, geographic origin, season of the year and diet. In order to obtain further information concerning individual patterns of expression of BJC isoforms, we have analyzed five individual *Bothrops jararaca* snake venoms. Venoms were collected at the same time from adult female snakes from the same geographic region. As expected, crude venoms showed a similar migration pattern on SDS-PAGE. BJC was purified by a previously described procedure, which includes an affinity chromatography step (PPACK-thrombin Sepharose). A slight variation in the amount of BJC obtained from individual venom samples was noticed. Inhibition of thrombin-induced platelet aggregation as well as migration pattern on SDS-PAGE (under reducing and non-reducing conditions) and isoelectric focusing varied considerably among BJC samples from the five snakes. The amino-terminal sequences (residues 1-34) of individual BJC samples were compared with the sequence deduced from isolated cDNAs encoding α and β chains of BJC. A high degree of homology was detected, although some residues differed from one sample to other. Altogether, the data confirmed the heterogeneity found for BJC purified from individual snakes. Thus, the results indicate that: 1) Individual specimens of *Bothrops jararaca* have different patterns of BJC isoform expression, and 2) it seems that genetic factors determine the variability found in BJC production.

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P-041Mo

MUSCARINIC SELECTIVE TOXINS IN *DENDROASPIS VIRIDIS* VENOM

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Dendroaspis angusticeps venom has been the most thoroughly investigated in the search for peptides that behave as muscarinic ligands: at least seven proteins (7kda) have been described (1, 2, 3). We have now isolated new toxins from venom of *D. viridis*, a phylogenetically related snake. We have characterised six fractions, four of which inhibited binding of ^{3}H-NMS to rat cerebral cortex membranes. One of these toxins, DvMT4, showed selectivity for the cloned m4 human muscarinic receptor subtype: affinity for m4 receptors was about 100 times greater than for m1 receptors (Ki of 19 nM and 1.7 μM, respectively), with little inhibition of binding to the other subtypes of muscarinic receptor. Inhibition curves in rat cerebral cortical membranes and brain slices were also performed. The data from each region showed the distribution of putative m4 receptors in the rat brain and fitted to competitive inhibition curves. A second fraction, DvMT3-4, showed high affinity for both m3 and m4 subtypes of receptor (Ki of 380 and 280 nM, resepectively). These experiments were compared with similar assays with MT1 and 2 from *D. angusticeps*, which showed high affinities for m1 and m4 receptors, although they are slightly higher for m1. From image analysis, it has been possible to compare the differential distribution and relative concentrations of muscarinic receptor subtypes. We expect that the discovery and characterisation of these and other additional muscarinic toxins with differential selectivities represent an advance in the library of ligands available for studying muscarinic receptor subtypes.

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P-042Mo

PARTIAL PRIMARY SEQUENCE AND CD SPECTRA STUDY OF A NEW NEUROTOXIN FROM *NAJA NAJA ATRA* VENOM

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Using ion-exchange chromatography, gel filtration, FPLC, and HPLC, we got two neurotoxic components from the venom of Naja naja atra (from the south of China). By PE ABD 476A Protein Sequencer, their partial N-terminal amino acid sequences had been determined. And using Jasco J-715 spectropolarimeter, we gained their CD spectra.

One of them has the same properties and amino acid sequence as Cobrotoxin (Cbt) reported. And the other, which we named it Cobrotoxin II (Cbt II), has a little lower toxic activity than that of Cbt, and it also has analgesic activity. By comparing its sequence with Cbt, Cbt II might be the result of the replacement of some amino acid residues at some positions: Thr11 to Ala, Thr15 to Lys, Gly20 to Glu, and Thr22 to Asn, and so on.

This may be an ordinary phenomenon in nature toxins. The cause might be the difference of habitat, season of milking, or distribution etc. So, more deeply research is needed.

The following is their partial amino acid sequences:

Cbt: H_2N-LECHNQQSSQTPTTTGCSGGETNCYKKRWRD..

CbtII: H_2N-LECHNQQSSQAPTTKGCSGEENNCYKKRWRD..

The CD spectra shows that they have very similar secondary structures. They all contain an anti-parallel three-chain β-sheet and several β-turns. We will compare their three dimensional structures by NMR later.

P-043Mo

A FIBRINOGEN-CLOTTING ENZYME FROM *TRIMERESURUS STEJNEGERI* VENOM, AND COMPARATIVE STUDY WITH OTHER VENOM PROTEASES

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The venom serine proteases which have been characterized so far are regarded as a family of proteins which specifically interact with different proteins on which they exert their physiological action. In the venom sample of a snake species, it often contains several protease homologues which may specifically act on different blood coagulation factors and show different biological functions. *Trimeresurus stejnegeri* venom, which contains TSV-PA (a specific plasminogen activator sharing 60-70% sequence homology with venom fibrinogen-clotting enzymes), also possesses fibrinogen-clotting activity *in vitro*. A fibrinogen-clotting enzyme (stejnobin) was purified to homogeneity. Comparative study of substrate specificities of stejnobin and other venom proteases was carried out on different substrates. Although stejnobin showed strong immunological cross-reaction with polyclonal antibodies raised against TSV-PA, it was interesting to observe that, unlike the case of TSV-PA, these antibodies did not inhibit the amidolytic and fibrinogen-clotting activities of stejnobin. The explanation of the great sequence homology of venom serine proteases verse their highly divergent substrate specificities (for protein substrates) is extremely significant in our understanding of protein structure-function relationship and the further protein reconstruction for medical purpose in future.

P-044Mo

SEASONAL VARIATION OF TOXICITY AND RESPONSABLE TOXINS IN TAIWANESE STARFISH *ASTROPECTEN SCOPARIUS*

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To elucidate the food chain of tetrodotoxin (TTX) in several gastropods containing TTX, the toxicity of marine animals inhabiting in the coastal waters of southern Taiwan was undertaken to examined. It was found that a starfish *Astropecten scoparius* showed lethal potency. Hence, paralytic toxicity was seasonally detected in every 10 specimens of the starfish *Astropecten scoparius* collected in Pingtung, Taiwan from December 1995 to November 1996. The highest toxicity of specimen, expressed as tetrodotoxin (TTX), was 5,938 mouse units (MU). The average toxicity of specimen was 1,679 MU and the respective toxicity was 133 and 99 MU/g in viscera and other parts. The toxin was partially purified by YM-2 membrane ultrafiltration and Bio-Gel P-2 column chromatography. TLC, electrophoresis, HPLC, UV spectrum and GC-MS analyses showed that the starfish toxin was composed of tetrodotoxin and anhydrotetrodotoxin.

P-045Mo

ISOLATION AND CHARACTERIZATION OF SEVEN POTASSIUM-CHANNEL PEPTIDE INHIBITORS FROM A CHINESE SCORPION VENOM

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With the goal of finding new K^+ channel blockers, we have investigated the venom of the Chinese scorpion *Buthus martensi*, by means of chromatographic fractionation. MALDI-TOF mass spectrometry fingerprinting of the C18-HPLC fractions allowed to identify 38 low molecular weight components of 3-4 kDa, only 7 of which were toxic to mice. The 48 other components are of higher molecular weight (6-8 kDa), and correspond, for most of them, to toxins acting on the Na^+ channels. The sequence of the 7 toxic short peptides was elucidated by Edman degradation: they are composed of a chain of 29 to 37 amino acid residues including 6 cysteine residues, displaying 40 to 90 % sequence identity with other known scorpion toxins. Due to their low representation in the venom (less than 0.05%), some of them were synthesized on solid phase, using Fmoc chemistry. Binding competition assays on rat brain membranes with radiolabeled specific probes of K^+ channels, and electrophysiological experiments using the voltage-gated K^+ channel Kv1.3 from rat brain, expressed in *Xenopus* oocytes, allowed to identify i) four toxins which bind to the small conductance Ca^{2+}-activated K^+ channels, one of them competed with the radiolabeled apamin, at IC_{50} of 20 pM, and the other at IC_{50}> 1µM; ii) three other toxins which are blockers of the Kv1.3 channel (IC_{50}s= 0.6-1.6 nM), and competed with iodinated kaliotoxin (IC_{50}s= 10-70 pM). Two among those toxins are structurally related to charybdotoxin (ChTX) and were tested in competition assay against iodinated ChTX on membranes from bovine aorta, in order to evaluate their potency to bind to the high conductance Ca^{2+}-activated K^+ channels.
Those new toxins interact with varying affinities with the K^+ channels tested, and display also multipoint mutations in their sequence as compared to the previously described scorpion toxins. They constitute new pharmacological probes for assessing the structure-activity relationships of this toxin family towards the various K^+ channels.

P-046Mo

A SINGLE STEP PURIFICATION METHOD FOR BASIC MYOTOXINS

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Snake venoms are complex mixtures of toxins, enzymes and peptides with a wide range of activities. Among these substances,myotoxins have been isolated from several venoms of different species. Many techniques have been described for isolating these compounds from crude venoms. In the present work, we describe a single step purification for some of these toxins. *Bothrops jararacussu* and *Crotalus durissus terrificus* venoms were purchased from Instituto Butantan. *Agkistrodon contortrix laticinctus* venom was a gift of Dr. C. Ownby and *Bothrops neuwiedii* venom was a gift of M. A. da Silva. All of these venoms were solubilized in 25mM sodium phosphate buffer, pH 7.8 and centrifuged prior to injection (200µl/run of a 10 mg/ml solution) in a Resource-S (Pharmacia) column, connected to a dual pump FPLC system. Buffer A and B were respectively 25 mM sodium phosphate and 25 mM sodium phosphate 2 M NaCl, both at pH 7.8. The flow rate during the whole run was of 3.5 ml/min. After an initial 10 ml wash with 7.5 % B, elution was started with a linear gradient (slope=1%) for 30 ml. The column was then regenerated with 10 ml 100% B buffer followed by a 10 ml A buffer wash. The complete procedure took about 20 minutes and preliminary tests indicate that it can be upscaled to 20 mg/run. All runs resulted in pure material, when assayed either by reverse phase chromatography on a C18 column eluted with a 0-60% acetonitrile gradient or by size exclusion HPLC.

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P-047Mo

NEW FAMILY OF POLYPEPTIDE TOXINS FROM ANT VENOMS

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A novel structural family of polypeptide toxins was discovered in the venom of tropical ants. Seven toxins called ectatomins (8000-9000 Da) were isolated from the venom of Peruvian ants *Ectatomma tuberculatum* and *Ectatomma quadridens*. A common structural feature of the isolated toxins is the presence of two highly homologous amphiphilic polypeptide chains (34-40 amino acid residues) connected or unconnected by a disulphide bond. The presence or absence of this disulphide bond provides subdividing all the toxins into two subgroups (Et an Eq). The amino acid sequence of four ectatomins was established and the secondary structure of three ectatomins was determined. The secondary structure of the toxins under study embraces over 65% of the α-helical conformation. Amino acid sequences of some ectatomins were confirmed by cloning and sequencing of the corresponding cDNAs. Each polypeptide chain of the toxin molecule was shown in the process to synthesize separately. Ectatomin Et-1 appeared to specifically affect cardiac L-type calcium currents. Both Et-1 and Et-2 formed cation-specific channels in cellular and artificial membranes at the concentration 10 nM displaying volt-ampere characteristics of the single channel. Ectatomin channel-forming properties might account for their toxic activity, but at the same time do not eliminate the intracellular action. These data indicate the existence of earlier unknown family of low molecular double-stranded polypeptide toxins.

P-048Mo

ISOLATION OF HEMORRHAGINS FROM VENOMS IN THE FAMILY VIPERIDAE

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Snakes belonging to the family Viperidae are among the most hemorrhagic. Snake venom is a complex mixture of toxins with many enzymatic activities which can create medical emergencies when humans are envenomated. The venom of the western diamondback rattlesnake (*Crotalus atrox*) has been reported to have seven known hemorrhagins. Hemorrhagic proteins are generally metalloproteinases which are responsible for the breakdown of tissue surrounding a snakebite. Many species of snakes from different geographical locations have venoms with different toxins but there are also common toxins found in different species of snakes. Metalloproteinases have important medical and biological implications in both pathological and normal physiological conditions. To better understand the utility and the mechanism of neutralization, purification of metalloproteinases is necessary. The purpose of this study was to compare six venom profiles by electrophoretic titration curves (ETC), high performance liquid chormatography (HPLC), and hemorrhagic assays. The venoms studied were *C. viridis lutosus, C. v. oreganus, C. v. viridis, C. atrox, Agkistrodon contortrix lacticinctus and A. piscivorus leucostoma.* The minimal hemorrhagic doses (MHDs) for the crude venoms were 2.1, 4.0, 1.5, 2.5, 4.2 and 3.9 µg, respectively. For each crude venom, an electrophoretic titration curve was used to determine optimal conditions for separation by ion exchange chromatography. A hemorrhagic assay was carried out on each fraction. An ETC was used to determine the pI of proteins in each fraction.

P-049Mo

THE ISOLATION OF METALLOPROTEINASES FROM THE VENOM OF THE NORTHERN BLACKTAIL RATTLESNAKE (*CROTALUS MOLOSSUS MOLOSSUS*)

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The Northern blacktail rattlesnake (*Crotalus molossus molossus*) is a member of the Viperidae family. The blacktails reside on the Edwards Plateau in which they seek foliaged ledges of limestone canyons. Although the blacktail is not found to be extremely aggressive, its venom is found to have a primary toxic effect of imparing coagulation, as well as a lethal potency some 79% that of the western diamondback (*C. atrox*). Venoms in the Viperidae family are among the most hemorrhagic of snake venoms. The hemorrhagins contained in the venom consist mainly of metalloproteinases. Metalloproteinases are largely responsible for the destruction of the endothelium cells in capillaries which allow blood to escape into surrounding tissue. In this study the venom of *C. m. molossus* was separated by anion exchange chromatography at pH 4.5. An electrophoretic titration curve (ETC) was used to predict the optimal conditions for the separation. Twenty-two fractions were collected, with fraction 7 having the most hemorrhagic activity. Fraction 7 was checked for purity using capillary electrophoresis (CE). Fraction 7 contained 5 peaks as determined by CE. An ETC for fraction 7 revealed 6 acidic bands with pIs ranging from 3.5 and 5. Further separation of fraction 7 was done using an anion exchange column at pH 8.0. Nine fractions were collected and only two fractions, 5 and 6, had hemorrhagic activity. The substrate specificity of these hemorrhagic fractions were examined using oxidized insulin ß chain and CE with a UV detector. The isolation of these metalloproteinases are essential for the production of monoclonal antibodies and the development of metallproteinase inhibitors.

P-050Mo

SEASONAL VARIATION OF PARALYTIC TOXICITY IN TAIWANESE CRAB *LOPHOZOZYMUS PICTOR* AND IDENTIFICATION OF THE RESPONSIBLE TOXIN

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Paralytic toxicity was detected by tetrodotoxin (TTX) bioassay in all 51 specimens of *Lophozozymus pictor* collected from March 1993 to January 1994 in northern Taiwan. It was found that the toxicity of this crab collected form October 1993 was higher than that of other months. The toxicity of viscera was higher than that of the cephalothorax and appendage. All specimens tested were toxic. The mean value of toxicity and the highest toxicity were 634 ± 80 (mean ± S.D.) mouse units per specimen (MU/specimen) and 3,874 MU/specimen, respectively. The toxin was partially purified from methanolic extract of *L. pictor* by ultrafiltration and Bio-Gel P-2 column chromatography. Electrophoresis, TLC, HPLC, UV spectrum and GC-MS analyses indicated that the toxin of *L. pictor* was mainly composed of TTX (88.9%), along with minor gonyautoxin 1,3 (11.1%).

P-051Mo

THE EFFECT OF *PANDINUS IMPERATOR* SCORPION VENOM ON THE K^+ CHANNELS OF HUMAN PERIPHERAL BLOOD LYMPHOCYTES

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Recently several novel K^+ channel blocking peptide toxins have been purified from the venom of the scorpion *Pandinus imperator* and the interaction of some of them with *Shaker* B K^+ channels has been demonstrated (T. Olamendi-Portugal et al. Biochem. J. (1996) 315, 977-981 and F.Gomez-Lagunas et al. J. Membrane Biol. 152:49 (1996)). In the present work we show that extracellular application of the *Pandinus imperator* whole venom and some of its tried peptide fractions markedly reduced whole-cell K^+ currents of human peripheral blood lymphocytes with half blocking concentrations in the range of 25 nM/ml - 0.5 nM/ml. The block of human lymphocyte K^+ channels by the whole Pi venom and its peptide toxin components was almost instantaneous and readily removable by perfusion of the cells with control extracellular solution. The present observations on human lymphocyte K^+ channels with fractions Pi1 and Pi2 are in line with the results of the above citations on *Shaker* B K^+ channels. The effect of the Pi peptide toxin components on the kinetic parameters of the human lymphocyte K^+ channels showed that activation and inactivation kinetics were both affected by the toxins, however, these changes were not as marked as the reduction of the whole-cell current amplitudes. Voltage dependence of steady-state inactivation and recovery from inactivation were not affected by the toxins. Flow cytometric measurements with the membrane potential sensitive oxonol dye revealed a depolarizing effect of Pi2 on human peripheral blood lymphocytes suggesting that the peptide toxin components of the venom of the scorpion *Pandinus imperator* may influence human lymphocyte activation.

P-052Mo

FURTHER CHARACTERIZATION OF PROTEINS FROM THE VENOM OF THE SCORPION *Pandinus imperator*, THAT CAUSE INHIBITION AND ACTIVATION OF RYANODINE BINDING TO Ca^{2+} CHANNELS

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We present the complete amino acid sequence of Imperatoxin A ($IpTx_a$), and Imperatoxin I ($IpTx_i$). Imperatoxin A, a 4 kDa peptide, specifically increased [^{3}H] ryanodine binding to skeletal (but not cardiac) RyRs with a Kd=6 nM (1). At concentrations higher than those needed to regulate RyRs, neither $IpTx_i$ nor $IpTx_a$ affected other Ca^{2+} channels of ion transporters of muscle and brain (2).Imperatoxin I, a 15 kDa protein, inhibited [3H] ryanodine binding to skeletal and cardiac ryanodine receptor with an apparent Kd=50 nM. The amino acid sequence of $IpTx_a$ shows no similarity to any scorpion toxin so far described, but shares some similarity to the amino acid sequence of Tx2-9 and Agelenin, two spider toxins that target neuronal P-type Ca^{2+} channels. The $IpTx_i$ is a heterodimeric protein with lipolytic action, a property that is only shared with ß-bungarotoxins. The large subunit of $IpTx_i$ was about 35% identical to PLA2 from bee (*Apis mellifera*) and lizard (*Heloderma horridum*) venoms. The small subunit has no significant similarity to any other known peptide.

References: (1). El-Hayek, R. *et al*, J. Biol. Chem. 270: 28696-28704 (1995); (2). Valdivia H.H, *et al* Proc. Natl. Acad.Sc.(USA) 89:12185-12189 (1992).

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P-053Mo

PHOSPHOLIPINS, A NEW FAMILY OF PHOSPHOLIPASE A_2 PRESENT IN THE VENOM OF SCORPIONS

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Three new heterodimeric phospholipases A_2 were purified and characterized from the venom of three different scorpions species. Partial peptidic amino acid sequence was obtained for two of them and the complete primary structure and nucleotidic sequence was obtained for the third one. Comparative analysis of the amino acid sequences of these *phospholipins*, as we proposed to call them, show that their catalytic sub-units do not belong to the current subclasses reported for phospholipases A_2's (PLA2). Imperatoxin I previously reported by our group (Zamudio *et al. J.Biol.Chem.* 272: 11886-11894, 1997), the honeybee and Heloderma PLA2 are the closest related structures, forming a peculiar cluster of sequences when a phylogenetic tree is drown. The small sub-units of unknown function, are covalently attached to the catalytic sub-units through a disulfide bridge, and may be playing a role as protease inhibitors. They all share same sequence similarities to *Cathepsin D Inhibitor* (Strukelj et al., *Nucleic Acids Res.* 18:4605, 1990). Since two of these phospholipins come from the same scorpion, a comparative analysis was conducted using both their nucleotide and amino acidic sequences. Despite the fact that 75% similarity was shown at the amino acid level, a very poor similarity was found at the nucleotide level, which could mind a distant gene duplication event.

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P-054Mo

ISOLATION AND CHARACTERIZATION OF A NOVEL K^+-CHANNEL BLOCKING TOXIN FROM THE VENOM OF THE SCORPION *Hadrurus aztecus*

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The scorpion *Hadrurus aztecus* is not dangerous to humans, and very little is known about the composition and function of its components. The soluble venom of this scorpion was applied to a Sephadex G-50 column resolving 7 fractions. Number III was subsequently separated by HPLC in a C_{18} reverse-phase given rise to at least 24 different sub-fractions. Component eluting at 24.90 minutes, provisionally labeled III.2, was obtained in homogeneous form and its *N*-terminal amino acid sequence was determined: Thr-Gly-Thr-Ser-Xxx-Ile-Ser-Pro-Lys-Gln... The effect of this peptide was assayed in cultured Sf9 cells infected with a recombinant baculovirus, capable of expressing the *Shaker*B K^+-channel. At a concentration near 130 nM about 60% reduction of the macroscopic currents was observed, under whole-cell patch-clamp, using borosilicate electrodes pulled to a 1.5 MOhms resistance. The effect was fully reversible. At higher concentration (around 0.7 μM) about 90% of the currents were reduced, with good recovery after washing. The blocking effect was not voltage-dependent.

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P-055Mo

HAEMORRHAGIN WITH FIBRINOLYTIC ACTIVITY FROM RUSSELL'S VIPER VENOM

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Russell's viper (RV) envenomation is a major health hazard in the Indian sub-continent. Among the major pathophysiological changes that occur haemorrhage and coagulopathies are most prominent. The chemical nature and pharmacological properties of RV venom haemorrhagins are still unclear. In this report we present data about a novel skin/ muscle haemorrhagic protein of 73 kD purified from RVV by chromatography on cm-sephadex c-50 followed by size exclusion HPLC. This protein was termed VRR 73. This haemorrhagic protein showed strong fibrinolytic activity independent of its haemorrhagic activity. Apart from these activities, it also showed moderate arginine esterolytic activity on benzoyl arginine p-nitroanilide (BAPNA).All three activities disappear with heating at 100° C, but only fibrinolytic and esterolytic activities reappear following cooling within an hour. Experiments with protease inhibitors and with antiserum showed that active sites of haemorrhagic and fibrinolytic actions are different. The fibrinolytic and esterolytic actions are probably dependent on a single active site.

P-056Mo

NEUTRALIZATION OF EDEMA, HEMORRHAGE AND MYONECROSIS INDUCED BY *CROTALID* VENOMS

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The venoms of *Agkistrodon contortrix laticinctis* (ACL) and *Crotalus viridis viridis* (CVV) cause severe local tissue damage such as edema, hemorrhage and myonecrosis. Antivenin (Av), though generally effective at reducing mortality, is rather ineffective at reducing morbidity unless incubated with the venom prior to injection. However, several substances, such as para-bromophenacyl bromide (PBPB), have been shown to antagonize the venom components responsible for this damage. In these experiments, mice were injected in the dorsolateral aspect of the gastrocnemius muscle with various combinations of venom, PBPB and Av to measure the neutralization capacity of the antagonists for ACL and CVV venoms. Edema was measured by determining increase in diameter of the leg, hemorrhage by determining the increase of hemoglobin locally, and myonecrosis by measuring the rise in creatine kinase levels in the blood. Edema was not neutralized by either antagonist alone, but was significantly reduced when both were used together. PBPB and Av tended to increase the hemorrhagic activity of the ACL venom, but halved the CVV induced hemorrhage. The antagonists showed a highly significant neutralization of ACL- and CVV-generated myonecrosis when used individually, and the neutralization was even greater when they were used together. Experiments in which venom and antagonist are injected separately are in progress.

P-057Tu

NUCLEOTIDE SEQUENCE AND EXPRESSION OF THE GENE CODING FOR HELOTHERMINE, A TOXIN FROM *Heloderma horridum horridum*.

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Helothermine (HLTX) is a toxin isolated from the venom of the Mexican Beaded Lizard *Heloderma horridum horridum*, which causes lethargy, partial paralysis and hypothermia in mice. It blocks ryanodine receptor channels incorporated into planar bilayers (Morrissette *et al.*, Biophys. J. 68:2280-2288, 1995). A cDNA clone coding for HLTX was obtained. It consists of 986 nucleotides including the 3' terminal region. The deduced primary structure of the mature peptide contains 283 amino acid residues closely packed by 16 cysteines, and was partially confirmed by Edman degradation of native HLTX and peptidic fragments obtained by enzymatic hydrolysis. Its amino acid sequence was shown to be similar to other proteins of the cysteine-rich secretory family (CRISP), isolated from human, mouse and rat testis as well as from salivary glands of male animals. Most of the eight disulfide bridges of HLTX and other CRISP proteins are situated at the C-terminal part of the molecule, strongly supporting the idea of two clear distinct structural domains in these molecules. The HLTX gene was expressed in *E. coli*, after introducing the clone into a pCSP105 vector (under the control of *lac* UV promoter), containing ampicillin-resistance and design to produce an hybrid recombinant protein with the product of the gene 9 from bacteriophage T3. By Western blot analysis it was demonstrated that the recombinant HLTX was recognized by antibodies raised against native HLTX. Purification of the recombinant HLTX has been attempted by means of an affinity resin prepared with antibodies. The final aim is to produce the two separate structural domains for functional analysis.

P-058Tu

ENCAPSULATION OF CROTOXIN INTO LIPOSOMES AND ITS ASSOCIATION WITH IMMUNOSTIMULANTS FOR THE PRODUCTION OF ANTIVENOM AGAINST *CROTALUS DURISSUS TERRIFICUS* SNAKE

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The present work aimed to evaluate the influence of lipid composition in the stability of liposomes obtained by the incorporation of crotoxin, the main toxic component of *Crotalus durissus terrificus* (Cdt) venom into Dehydration Rehydration Vesicles (DRV/crotoxin). The immunoadjuvant effect of DRV/crotoxin associated with immunostimulants; lipopolysaccharide from *Escherichia coli* (LPS) and monophosphoryl lipid A from *Salmonella minnesota* (MPLA), has been investigated.

DRV/crotoxin made with sphingomyelin (DRV/Sm) or dipalmitoylphosphatidylcholine (DRV/Dppc) (D stereoisomer), phospholipids which are inative as substrates for snake venoms' phospholipase A_2 activity, were shown to stimulate the humoral immune response. The association of the DRV/Sm preparation with LPS (DRV/Sm/LPS) was found to enhance antibody production. Vaccination of mice with DRV/Sm/LPS (3 doses of 6 µg of crotoxin/mice) conferred protection in 80% of animals against a dose of Cdt venom equivalent to 8 LD_{50}. Incorporation of MPLA into DRV/Sm did not increase significantly either antibody titre, or protection against Cdt venom. Crotoxin encapsulated into DRV made with Dppc (L stereoisomer), a substrate for venom phospholipase A_2 activity, was shown to be less stable than liposomes composed of Sm or D-Dppc. On the other hand, vaccination of mice with DRV/L-Dppc associated with LPS or MPLA, produced a high and similar immune response with respect to antibody titre and protection.

Our data indicates that crotoxin encapsulated into a liposome composed of L-Dppc, a synthetic phospholipid, in association with a non toxic immunostimulant MPLA, is able to induce a high level of immune response against Cdt venom.

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P-059Tu

EFFECT OF DETOXIFICATION CAUSED BY THE INCORPORATION OF *BOTHROPS JARARACA* VENOM INTO DEHYDRATION REHYDRATION VESICLES AND EVALUATION OF LIPOSOME STABILITY

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The commercial production of antisera against snake venoms uses whole venoms as antigens and Freund's Adjuvant as immunoadjuvant. The search for venom detoxification and the development of a potent and safe immunoadjuvant system are important aspects of research to improve immunization efficiency.

The present work aimed at evaluating the effect of liposome incorporation in the reduction of venom toxicity. A batch of *Bothrops jararaca* venom with a subcutaneous LD_{50} of 90 µg/20 g mouse (68-122 µg/20 g; 95% confidence limits) was used to evaluate the reduction of toxicity by either the emulsification of venom with Freund's Adjuvant (FCA/*Bj*) or its incorporation into Dehydration Rehydration Vesicles (DRV/*Bj*), made with sphingomyelin, a phospholipid which is inactive as a substrate for snake venom phospholipase A_2 activity. DRV/*Bj* was found to be less toxic than FCA/*Bj*: liposome preparation was not toxic to mice when sc inoculated at 6 times its LD_{50} while the same dose of venom emulsified with Freund's Adjuvant killed all inoculated animals. DRV/*Bj* was shown to be very stable. Protein released from vesicles after one week incubation at 4, 25 or 37°C, was less than 4% of the original incorporated venom. The released protein showed a similar profile in polyacrylamide gels as the native venom which is evidence that encapsulation into liposomes did not alter the venom's integrity. The encapsulation efficiency of native venom was shown to depend on the initial venom concentration, with a maximum of 26% incorporation, when 1.25 g/L was used. We are working on other parameters to increase venom incorporation. Our data indicates that DRV/*Bj* should be investigated as a potential immunoadjuvant in the immunization protocol to produce antiserum against *Bothrops jararaca* venom.

Financial support: This research was supported by Fundação de Amparo a Pesquisa de Minas Gerais (FAPEMIG).

P-060Tu

DETOXIFICATION WITH FORMALDEHYDE OF VENEZUELAN *TITYUS* GENUS SCORPION VENOMS. IMMUNOGENICITY OF TOXOID AS COMPARED TO CRUDE VENOM.

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Scorpion venom was obtained by electric stimulation. The s.c. LD_{50} in 1h was determined in mice C57/B (♀, 14 to 18 g) using the method of Dixon and Mood (1948). The LD_{50}s were: *T. discrepans*, 8.8 (8.3, 9.4) mg/kg; *T. ivic-nancor n.sp.* (González-Sponga), 8.9 (8.4, 9.4) mg/kg and *T. caripitensis n.sp.* (Quiroga), 14.6 (13.7, 15.4) mg/kg mice. Ten milligrams of venom were detoxified (Sadahiro ,1971). Eight mg/kg of this material were injected s.c. to mice 3 times, at 21 day intervals. The first two doses were emulsified with Freund's incomplete adjuvant. The formaldehyde-treated venom (F-TV) was contrasted with venom freshly diluted (FDV), and with venom processed as explained, but without formaldehyde (VWF). A control group of mice was injected with distilled water only. Blood was drawn from the tail 100 days after the third dose. Serum was centrifuged, pooled and divided into 0.2 ml aliquots kept at -20°C until use. An 1:200 dilution was assayed by enzyme linked immunosorbent assays (ELISA). Immediately after bleeding mice were challenged with 1 s.c. LD_{50} of venom. Treatment with F-TV had no adverse effects and was efficient at inducing immunity. Treatment with FDV, and VWF had variable toxic effects (11% of death with FDV), specially with the 1st dose. ELISA proved that F-TV was more immunogenic [1.86 (1.79, 1.92) A at 490 nm] than FDV [1.35 (1.28, 1.41) A] or VWF [0.83 (0.74, 0.93) A]. Yet, F-TV and FDV were equally efficient at protecting mice against the venom.

P-061Tu

IMPORTANCE OF ALPHA-MACROGLOBULINS IN THE CONTROL OF HEMORRHAGIC METALLOPROTEINASE ENVENOMATION

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The hemorrhagic/fibrinolytic metalloproteinase in *Bothrops jararaca* venom, jararafibrase I, is inhibited by human and rat sera. We found that in human serum, alpha2-macroglobulin was responsible for the inhibition of jararafibrase I while in rat serum, alpha1-macroglobulin and murinoglobulin were responsible for the inhibition. To elucidate the role of such macroglobulins, two groups of rats were pretreated with methylamine to reduce the macroglobulin activity to less than 20% of the normal value. After the pretreatment, one group of rats was given 50 µg/kg of purified jararafibrase I intravenously and the plasma concentration of the enzyme was monitored by an ELISA technique. The methylamine pretreatment appreciably lowered the disappearance rate of intravenously administered jararafibrase I from the plasma. Even at 2 hrs after administration, more than 3% of the administered jararafibrase I still remained in the plasma, whereas only 0.1% remained in the non-pretreated control group. The other pretreated group of rats was given 50 µl of *B. jararaca* crude venom (2 mg/ml) intradermally into the dorsal skin. After 18 hrs, the areas of hemorrhage were measured. The area in the methylamine-pretreated group was almost 3 time wider than that in the saline-infused control rats. The present findings suggest that alpha-macroglobulins played a very important role not only in the excretion of hemorrhagic metalloproteinase from the plasma but also in the prevention of local hemorrhage and necrosis.

P-062Tu

NEUTRALIZING CAPACITY OF Gallus domesticus SERUM AGAINST RUSSELL'S VIPER VENOM

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Several animals exist in nature which are known to be snake venom resistant. Some of the reported species of animal are snake, mongoose, hedgehog, woodrat etc. Many Indian bird species like peacock, owl, eagle, wild hen are known to kill and eat snakes. Venom resistant properties of these species have not been reported earlier. The present investigation explored the neutralizing capacity of hen (Gallus domesticus) serum against Vipera russellii venom induced lethality, haemorrhagic and defibrinogenating activity in experimental animals. The hen (Gallus domesticus) serum effectively neutralized the Viper venom induced lethal, haemorrhagic and defibrinogenating action. The Serum neutralized $2LD_{50}$ dose of lethal and 3MHD of haemorrhagic action of Viper venom. The serum was further isolated and purified on DE-50 Sephadex column chromatography. The serum factor seems to be a high molecular weight protein and was found to be active against Viper venom induced haemorrhagic action. The above observation may add new information in the area of snake venom research.

P-063Tu

ANTITOXIN TO HOLOCYCLOTOXIN, THE PARALYZING TOXIN OF THE AUSTRALIAN PARALYSIS TICK, *Ixodes holocyclus* AND ITS EVALUATION

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The Australian paralysis tick, *Ixodes holocyclus* may cause paralysis and death in domestic pets, livestock and humans. An antitoxin raised in hyperimmune canines has been available for many years. [Although monoclonal antibodies have been raised to **holocyclotoxin,** these have proved to be disappointing in terms of neutralizing potency (Stone, *et al.*, unpublished)]. The canine antitoxin has been standardized by means of a mouse bioassay (Stone, Cowie, Kerr and Binnington, 1982). In this method, **holocyclotoxin** is serially diluted and incubated with a standard volume of antitoxin for 45 min at 37°C, and the residual toxin assayed by injection into 4-5g mice. These are incubated at 30°C for 24hr and paralysis rated quantitatively over a 1-10 range. Biometric analysis of data allows neutralizing antibody titres to be rated in arbitrary antitoxin units/ml.

The method has been modified in accordance with more common practice which calls for serial dilution of antitoxin titrated against a standard dose of **holocyclotoxin.** A minimum titre of 500 "new" units/ml is required for acceptable registration. Progress has been made in evaluation of the potency of the antitoxin by means of an EIA method and correlation with the bioassay-derived titres is being investigated.

Stone, B.F., Cowie, M.R., Kerr, J.D. and Binnington, K.C. (1982). Improved toxin/antitoxin assays for studies on the Australian paralysis tick Ixodes holocyclus. Aust. J. Exp. Biol. Med. Sci. 60: 309-318.

P-064Tu

ELISA FOR THE DETECTION OF VENOMS IN EXPERIMENTAL AND CLINICAL ENVENOMING BY *Loxosceles intermedia* SPIDERS

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Curitiba is the capital of Paraná State, Southern of Brazil, and for unknown reasons has registered an increasing incidence of bites by the spider *Loxosceles intermedia* during the last ten years. Health Office reports indicate 100 cases in 1986, 923 cases in 1989-1990, 1200 cases in 1992 (3), and more than 3000 cases in 1995 and these figures are much above the average number of accidents with spiders reported yearly in Brazil.Enzyme linked immunosorbent assays (ELISA) were developed to detect specific antigens from *Loxosceles* spiders venom. Hyperimmune horse anti-*Loxosceles intermedia* IgGs were prepared by immunoaffinity chromatography and used to set up a sandwich-type ELISA. The specificity of the assay was demonstrated by its capacity to correctly discriminate the circulating antigens in mice that were experimentally inoculated with *L. intermedia* venom from those inoculated with *L. laeta* and *Phoneutria nigriventer* spider venoms, *Tityus serrulatus* scorpion venom and *Bothrops jararaca, Crotalus durissus terrificus, Lachesis muta muta* and *Micrurus frontalis* snake venoms. Measurable absorbance signals were obtained with 0.8 ng of venom per assay. The ELISA also detected antigens in the sera of patients envenomed by *L. intermedia*. Therefore, after standardization for clinical use this ELISA may be a valuable tool for clinicians and epidemiologists due to its sensitivity and specificity.

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P-065Tu

PREPARATION OF A TRANSUDATE FROM *Bothrops jararaca*, USING A SURGICAL IMPLANT, THAT CONTAINS ANTIHEMORRHAGIC ACTIVITY AGAINST THE AUTOLOGOUS VENOM.

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A new methodology was developed for preparation of the antihemorrhagic factor with good yield avoiding animal suffering. Implants were inserted in the abdominal cavity and eight weeks after surgery the first transudate was collected. *B. jararaca* serum was obtained by cardiac and caudal. vein punction. The antihemorrhagic assay was performed on rats. Electrophoretic analyses were run under non-denaturing conditions and SDS-PAGE. Phenyl-5PW chromatography was run employing a decreasing linear ammonium sulphate gradient. The transudate was analyzed comparatively to the serum. In the antihemorrhagic assay, serum inhibited one minimal hemorrhagic dose (MHD) of *B. jararaca* venom in a 9 to 1 ratio (serum:venom, w/w). On the other hand, for the transudate a 6 to 1 ratio was sufficient to obtain total inhibition of one MHD. The electrophoretic separation of transudate and serum proteins showed the same band pattern in native as well as in denaturing and reducing conditions, suggesting that these two samples possess similar proteic heterogeneity. In both, bands in the 56kDa region could be observed, being this last the molecular mass of an antihemorrhagic factor already purified by Tanizaki *et al.* (Toxicon, 29:673-681, 1991). The chromatographic profiles of the two samples by hydrophobic interaction chromatography also showed similarity. The technique employed for the active transudate preparation may substitute the traditional serum obtention, as starting raw material for the purification of the antihemorrhagic factor, avoiding animal suffering and eventual sacrifice. This technique may be employed in the study of other snake species.

Financial support: CAPES, CNPq, FAPERJ, FINEP and PAPES-FIOCRUZ.

P-066Tu

NEW METHODOLOGY FOR THE PRODUCTION OF THE ANTIBOTHROPIC COMPLEX FROM *DIDELPHIS MARSUPIALIS* (OPOSSUM).

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The antibothropic complex from *D.marsupialis* (ABC) was generated inside perforated hollow plastic golf balls which were surgically implanted subcutaneously in anesthetized opossums, a technique originally described for the production of polyclonal antibodies (*Infect.Immun.*10:458,1974). Two months after the implantation of the balls, approximately 15 ml of seromatous fluid (SF) (25 mg total protein/ml) were recovered monthly. Opossum serum as well as SF showed similar SDS-PAGE profiles and antihemorrhagic potencies against *Bothrops jararaca* snake venom (Bjv). The presence of ABC in SF was confirmed by ELISA and immunoblotting assays, using rabbit protein A-purified polyclonal antibodies raised against ABC isolated from opossum serum. ABC isolated from SF or from serum by ion-exchange chromatography on DEAE-Sephacel showed identical chromathographic and eletrophoretic profiles. ABC from both sources displayed very similar antihemorrhagic and anticascinolytic activities against Bjv. We concluded that this new methodology is very suitable for continuously obtaining opossum ABC in large scale in an easier way and without animal suffering.

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P-067Tu

CHARACTERIZATION OF THE 48 kDa SUBUNIT FROM THE ANTIBOTHROPIC COMPLEX ISOLATED FROM *DIDELPHIS MARSUPIALIS* (OPOSSUM) SERUM.

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The isolation and characterization of the antibothropic complex (ABC) from *Didelphis marsupialis* serum have been previously described (*Toxicon* 32:1237,1994). Its 48 kDa subunit (S48) was purified by Phenyl Sepharose chromatography and was homogeneous on SDS-PAGE. S48 was analysed by HPLC gel filtration chromatography on a Protein Pak 300 SW column and a main peak of 77 kDa was detected, indicating a dimeric nature. S48 was submited to isoelectric focusing, its pI being lower than 4.5. Using the dry weight method, the subunit's calculated extinction coefficient was 66,882 M^{-1} cm^{-1}. S48 is a glycoprotein, as indicated by periodic acid-Schiff reagent staining. After chemical deglycosylation with anhydrous trifluoromethanesulfonic acid, its molecular mass was reduced by 25%. Amino acid composition of S48 was determined by acid hydrolysis for 24 and 48 hours, followed by ion-exchange chromatography and post-column derivatization with ninhydrin. High contents of hydrophobic residues (40%) were detected. On molar basis, 8 cysteines and 4 methionines were found. Same inhibition rates of *Bothrops jararaca* venom (Bjv) caseinolytic activity were achieved with similar amounts of ABC and S48. Bjv lethal and hemorrhagic activities were more effectively inhibited by ABC.

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P-068Tu

PRODUCTION IN *E. COLI* OF SCORPION TOXINS FROM *ANDROCTONUS AUSTRALIS* SCORPION VENOM

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cDNAs encoding different toxins from the venom of the North African scorpion *Androctonus australis, i. e*: 1) the short polypeptide blocker of K^+ channels, KTX2; 2) the α-type toxins acting of the voltage-sensitive Na^+ channels AaH I, AaH II and AaH III; were expressed in the periplasmic space of *Escherichia coli*, as fusion proteins with the maltose binding protein followed by the recognition site for factor Xa or enterokinase preceding the first amino acid residue of the toxin. The *in vivo* fully refolded recombinant toxins were compared to the native toxins according to chemical and biological criteria. A non amidated rAaH II, a C-extended rAaH II analogs and a N-extended analog of KTX2 exhibiting three additional residues were also obtained. The N-extended analog of KTX2 had 1000-fold less affinity for the [^{125}I]-KTX binding site on rat brain synaptosomes than KTX2. On the contrary, the C-terminal modified rAaH II showed activities practically indistinguishable from AaH II. The fusion proteins were also used as non toxic antigens to generate antibodies in rabbit. Using radioimmunoassaies, it was shown that these antibodies were able to recognize their specific [^{125}I]-toxin with affinities in the 0.1 nM range. Moreover, *in vitro*, the antibodies raised against the fusion-KTX2 were able to inhibit the binding of [^{125}I]-KTX to its receptor on rat brain synaptosomes.

P-069Tu

USEFULNESS OF A NON-TOXIC ANALOG TO ELICIT NEUTRALIZING POLY AND MONOCLONAL ANTIBODIES AGAINST SCORPION TOXIN

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Scorpion venoms contain toxins that act on ion channels and are likely responsible for the noxious effects observed when people are stung by scorpions. The study of the neutralization of these molecules is a purposeful challenge and producing monoclonal antibodies (mAbs) should provide powerful tools. Venom from *Androctonus australis hector* , a North african scorpion contains several potent toxins. We synthesized a non-toxic analog of the toxin II (Aah II), the (abu)8-AahII which was demonstrated very useful in numerous applications. First this analog was able to neutralize the native toxin and to confer protection against scorpion toxin in animal models (Zenouaki I., Kharrat R., Sabatier J-M., Devaux C., Karoui H., Van Rietschoten J., El Ayeb M. and Rochat H. 1997, Vaccine, 15: 187-194). Second we used (abu)8-AahII to produce several monoclonal antibodies which recognize and neutralize the native toxin II. Their binding sites on toxin were delineated by ELISA using sets of peptides spanning the entire sequence of AahII. Then, recently we used (abu)8-AahII as a protein carrier to obtain mAbs against another toxin (AahI) which was anable to elicit antibodies in BALB/C mice. Four clones are now under investigation.

P-070Tu

IMMUNOLOGICAL CROSS REACTIVITY AMONG VENOMS OF TRIMERESURUS SPECIES IN THAILAND AND JAPAN

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Venoms of Malaysian Trimeresurus species have been shown to contain common antigens (Tan and Lim; 1993). Hemorrhagic activity of Japanese Habu (T. flavoviridis) venom was inhibited by commercial polyspecific antivenoms for Crotalidae (Mebs et al., 1988).

Present study has been undertaken to see immunological cross reactivity among venoms of Trimeresurus species in Thailand and Japan. Results of SDS-PAGE and immunoblotting analysis demonstrated that Thai and Japanese Trimeresurus venoms contain many cross reactive protein components. Considerable cross neutralization was also observed: hemorrhagic activity of Thai Trimeresurus venoms was neutralized by Habu monospecific antivenom, and vice versa. Thai Trimeresurus antivenom was equally potent in neutralizing the hemorrhagic activity of Japanese Habu venom.

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P-071Tu

DEVELOPMENT OF REVERSED PASSIVE LATEX AGGLUTINATION FOR DETECTION OF THAI COBRA (NAJA KAOUTHIA) VENOM

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A simple and rapid technique detecting Thai cobra (N. kaouthia) venom has been developed by using the reversed passive latex agglutination. Three types of commercial latex preparation were sensitized with different concentration of affinity purified horse IgG raised to Thai cobra venom. With the higher concentration of IgG, the higher sensitivity. Particle size and latex concentration affected the sensitivity. The best condition to detect cobra venom was established. The minimum venom concentrations to detect cobra venom in diluent solution, human plasma and serum in 30 min were 25, 12.5 and 50 ng/ml, respectively. Cross reaction with heterologous venoms was also examined. Positive reaction was found with king cobra venom at 1,600 ng/ml. On the other hand, a strong reaction was found with spitting cobra (N. siamensis) venom at 400 ng/ml. The results suggest that the detection method established in this study is useful for diagnosis of cobra-bite victims.

P-072Tu

COMPARATIVE STUDY OF BOTHROJARACIN, WITH HOMOLOGOUS PROTEINS

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Bothrojaracin is an antagonist of thrombin purified from *Bothrops jararaca* venom (MW=27Kda). The mechanism of inhibitory activity is similar to hirudin, a peptide purified from *Hirudus medicinallis* by the formation of a reversible complex with thrombin. The N-terminal sequence (27 amino-acids) of bothrojaracin is homologous to proteins with different biological activities isolated from same venom (Botrocetin, Lectin-BJL and Factor IX-X inhibitor) and from other venoms (Convulxin). In this work, we compare antigenic cross-reactivity obtained with proteins known as homologous (botrocetin, lectin-BJL, convulxin) or not (from *Naja moçambique moçambique* and *Naja naja kaouthia*) to bothrojaracin using anti-bothrojaracin serum raised against native and denaturated form as well as with anti-convulxin serum.

Immunodiffusion assay with these proteins shows partial identity between botrocetin and bothrojaracin. Western-blotting assays showed that just lectins were not recognized by anti-bothrojaracin sera. We observed with ELISA assays that convulxin and botrocetin are immunologically similar to bothrojaracin denaturated and native form. Factor IX/X inhibitor was only recognized by anti-bothrojaracin raised against denaturated form. Bothrojaracin and convulxin were then analyzed with anti-convulxin serum. Results with western blotting and ELISA assay showed that anti-convulxin serum also recognized bothrojaracin molecules but with lower potency than anti-bothrojaracin serum recognized convulxin. These results suggests that convulxin and botrocetin present some epitopes which are similar or identical to those in native structure of bothrojaracin. No immunological relationship was verified using this antiserum between bothrojaracin and lectins. Although these proteins present some parts of their sequences similar with bothrojaracin, they are not structurally similar with bothrojaracin epitopes.

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P-073Tu

A FIELD TRIAL WITH F(ab')$_2$ ANTIVENOM IN NORTH CAMEROON

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A trial has been performed in North Cameroon to measure the tolerance and the efficiency of a purified F(ab')$_2$ polyvalent antivenom. The study involved eight health centers. However, 97% of patients were included by five of them. The antivenom was administered through intravenous route by infusion of 2 vials renewed every 2 or 4 hours until recovery. The supervision lasted at least 5 days and patients had to be re-examined three weeks after admission to evaluate the tolerance.
The trial involved 223 subjects who presented an actual envenomation. *Echis ocellatus* was responsible for most of the envenomations. More than 80% of patients presented a haemorragic syndrome at the time of admission.
In 17 patients (7.6%), it has been observed an adverse event whose causal relationship to the antivenom was considered as possible or probable. Half of these events occurred within 30 minutes following the antivenom administration. One severe early adverse event (0.4%) has been recorded. One patient (0.6% out of the 165 patients re-examined three weeks after the treatment) presented a serum disease. The recovery has been obtained for 213 subjects without any major sequel (95.5%). Three patients (1.3%, *i.e.* more than four fold fewer than the letality recorded in these centres before the trial) deceased from haemorragic complications due to delayed antivenom administration. Seven other patients (3.1%) withdrew from the trial before the last visit.
This trial allowed us to validate clinic and biological indicators useful for the supervision of treatment. They will allow to improve the therapeutic protocol, notably concerning doses. The antivenom administered by intravenous route appeared to be well tolerated, even at high doses, and efficient.

P-074Tu

TREATMENT OF SNAKEBITE BY TRYPSIN AND CHYMOTRYPSIN

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Locally injected lethal dose of Chinese *Elapidae* and *Hydrophiidae* snake venoms into mice or dogs, then immediately or after 10 min injected trypsin and chymotrypsin (100 U/per animal) at the same spot, the experimental animals were all survived. The efficiency was the same as antisera. The longer the interval, the lower the result. The treatment efficiency of antisera was higher than trypsin method if the time interval between 20-30 mins. Over 40 mins, none had effect. Locally injected Chinese *Viperidae* and *Crotalidae* venom, then immediately or after 40 mins to treat, the efficiency was 50-75%, lower than antisera, which was 60-100%.
Circular injected trypsin and chymotrypsin at the biting spot to block and decompose the snake venom proteins, combined other treatment methods, such as taking "Yunnan snakebite drug" and control fluid transfusion etc. We have cured over 4,000 cases of various snakebite patient with the efficiency of 99.8%.
For local necrosis, besides adopting the above methods, incising and cleaning the dead tissue, coating or injecting EDTA to the necrosis area should also be used as early as possible. Once the fresh tissue began to grow, skin-transplant should be done quickly to minimize the necrosis sequela.
Compared this treatment method with classic antisera method, the efficiency of both methods is similar. but this method has characteristics of broad spectrum, smaller side-effects, easy to conduct and lower necrosis rate, and provides a new pathway for snakebite treatment.

P-075Tu

Studies on some protective manoeuvres in mulgotoxin induced rhabdomyolysis and renal damage.

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Mulgotoxin, a myotoxic phospholipase A_2 from the venom of *Pseudechis australis* induced myonecrosis and renal damage in mice. Serum creatine kinase (CK), creatinine and urea showed marked elevation, indicative of rhabdomyolysis with acute renal failure. Nuclear microscopic (NM) studies of necrotic muscle fibres showed early (1h & 6h) elevation of total Ca (400%-600%) and Na (500%) levels. Protective effects of some drugs were done using verapamil (Ca^{2+} channel blocker), dantrolene (inhibitor of Ca^{2+} release from sarcoplasmic reticulum [SR]), ouabain (inhibitor of Na-K ATPase), deferoxamine (iron chelator and anti-oxidant) and anti-mulgotoxin. Two groups of mice were studied using three different dosage (1mg, 2.5 mg and 5 mg/kg; deferoxamine at 300 mg/kg). The first groups were pretreated i.p. and received an additional i.m. dose immediately after injection of the toxin, the second group received a single i.m. dose after injection of the toxin. Effects of acidification (NH_4Cl) and alkalinization ($NaHCO_3$) on mulgotoxin treated mice were also done. Light microscopy, NM and serum CK levels showed that dantrolene offered significant protection from myonecrosis in mice pretreated with it. Similarly alkalinization of urine showed marked protection from myoglobinuric renal damage as revealed by LM and serum creatine and urea levels. Anti-mulgotoxin showed only 50% protection (by CK levels) and other drugs were not protective. Thus, Ca^{2+} release from SR may play a key role in triggering myonecrosis and dantrolene can be explored for therapeutic potential in controlling myonecrois.

P-076Tu

HUMAN HIGH MOLECULAR WEIGHT KININOGEN INHIBITS HEMORRHAGIC ACTIVITY OF *BOTHROPS JARARACA* (Bj) VENOM.

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Human and Bj HMW kininogens are able to inhibit activities of the Bj venom in which metalloproteinases are involved (previous results obtained in our laboratory). The goal of the present work was to study the mechanism of this inhibition, focusing a possible complex formation, as well as a possible influence of divalent ions. Complexes between kininogen and components of the venom were not detected by FPLC or PAGE. However, when kininogen was pre-incubated with Ca^{2+} or Zn^{2+} and then incubated with the Bj venom, the inhibition of the hemorrhage was partially reverted. Using two commercial human kininogens (single chain and double chain high molecular weight kininogens, Enzyme Research- USA) significant inhibition of hemorrhage was observed only with single chain kininogen, indicating a specific domain responsible for this effect. These data indicate that kininogen inhibits metalloproteinases of the Bj venom through competition for divalent ions and the domain 4 of the kininogen seems to be involved.

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P-077Tu

CHARACTERIZATION OF *Micrurus frontalis altirostris* VENOM.

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In Rio Grande do Sul State; Brazil, human accidents caused by the snake ***Micrurus frontalis altirostris***, are rare, but fatal. The purpose of the present study was to characterize some biological activities of this venom. The venom of ***Micrurus frontalis altirostris*** did not induce hemorrhage, dermonecrotic damage or coagulant activity on horse plasma "**in vitro**". In addition, low proteolitic activity was observed when tested on caseín. However, high indirect hemolytic activity was demonstrated. The peak of edematogenic activity occured 30 min. after venom inocculation, and the minimum edematogenic dose was 0,273 µg/animal. The venom toxicity (DL_{50}= 0,255 mg/kg), may be considered high, when compared to other ***Micrurus*** species (***M. ibiboboca*** DL_{50}= 1,48 mg/kg and ***M. lemniscatus*** DL_{50}= 3,87 mg/kg). Interestingly, the commercial ***Micrurus*** antivenom produced by Instituto Butantan could not neutralize the lethal activity of ***Micrurus frontalis altirostris*** venom, when tested by ***"in vivo"*** venom neutralization.

P-078Tu

SERUM LEVELS OF CYTOKINES IN PATIENTS ENVENOMED BY *TITYUS SERRULATUS* SCORPION STING.

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Seventeen patients stung by *Tityus serrulatus* scorpion were studied after their admission to Hospital João XXIII in Belo Horizonte, Minas Gerais, Brazil. The patients were classified as mild (n=6), moderate (n=10) and severe cases (n=1) according to the severity of the clinical manifestations. Patients who presented only pain at the site of the sting were classified as mild cases. Moderate cases presented in addition to local pain at least one of the following manifestations: vomiting, psychomotor agitation, prostration, sweating, tachypnea, tachycardia and mild arterial hypertension. The severe case presented in addition to the aforementioned manifestations cardiac failure, pulmonary oedema and shock. Venous blood was sampled for biochemical and haematological analysis (moderate and severe cases) and for IL-1, IL-6, IL-10, TNF-α, IFN-γ and GM-CSF enzyme-linked immunosorbent assays at the time of hospital admission (mild and moderate cases), at 6hr (moderate and severe cases) and at 12hr, 18hr, 20hr and 120hr later after antivenom administration. Blood samples from 10 healthy volunteers were used as control. Data from this study show an increase in the circulating levels of IL-1 and IL-6 in the patients with mild, moderate and severe envenoming when compared to the cytokine levels measured in tle sera of the normal volunteers. The patient with severe envenoming also presented high levels of IL-10 and GM-CFS. Our data suggest that a systemic inflammatory response-like syndrome could be triggered in patients stung by *Tityus serrulatus* and that the release of cytokines could play an important role in the pathophysiology of severe scorpion envenoming. Acknowledgements: CNPq and FAPEMIG.

P-079Tu

TOURNIQUET INEFFECTIVENESS TO REDUCE THE SEVERITY OF ENVENOMING AFTER *CROTALUS DURISSUS* SNAKE BITE IN MINAS GERAIS, BRAZIL.

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This study reports the effects of tourniquet application on the course of envenoming after *Crotalus durissus* snakebites in the rural areas surrounding Belo Horizonte, Minas Gerais, Brazil. Clinical and laboratory data from 97 patients admitted to Hospital João XXIII, Belo Horizonte, were recorded on a proforma. The time elapsed between the bite and application of the tourniquet, how long it was left in place, tightness of the tourniquet and the effects of its removal on symptoms were not available. Blood was sampled for biochemical and haematological analysis before antivenom treatment and for whole venom and crotoxin assays (ELISA) after the release of the tourniquet. The patients were treated with 100-200 ml of *Crotalus durissus* antivenom. Forty-five (46.4%) of the patients were included into the tourniquet group and 52 (53.6%) into the non-tourniquet group. The gender, age, time elapsed between bite and hospital admission, dose of antivenom and the frequency of palpebral ptosis did not differ between the two groups. Maximum plasma creatine kinase enzyme activity and partial thromboplastin time, plasma whole venom and crotoxin concentrations and the frequency of complications such as acute renal and respiratory failure, local oedema and number of deaths did not also differ between both groups. Data from this study show the ineffectiveness of tourniquet applied by the patients in the fields to reduce the severity of *Crotalus durissus* envenoming.
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P-080Tu

THE DEVELOPMENT OF THE FIVE STEP WESTERN BLOT

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Most snakes and a few warm-blooded animal sera neutralizes the hemorrhagic activity of snake venoms. Antihemorrhagins in woodrat (*Neotoma micropus*) and opossum (*Didelphis virginiana*) sera neutralize all hemorrhagins in snake venoms tested. A modified western blot (five step western blot) was developed to detect the presence of antihemorrhagic activity in resistant animals. In the five step western blot, *C. atrox* venom is separated by native-PAGE electrophoresis, transferred to immobilon membrane and then reacted with crude opossum serum. The antihemorrhagins in opossum serum bind to hemorrhagins in snake venom which are then reacted with a monoclonal antibody (OPO-SS) specific for the antihemorrhagins. Goat anti-mouse (IgG) antibody conjugated with horseradish peroxidase and 4-Chloro-1-Naphthol were added to the blot to detect the binding of antihemorrhagins to hemorrhagins. The five step western blot has two well defined areas and a third diffused area which could be representative of the seven hemorrhagins in *C. atrox* venom. The opossum antihemorrhagins form a complex by binding with *C. atrox* hemorrhagins separated by electrophoresis. Hemorrhagins have common amino acid sequences; and could therefore, have a common binding site for antihemorrhagins found in woodrat and opossum sera. The five step western blot will be a useful *in vitro* assay for (1) detecting antihemorrhagic activity in animal sera without using live animals, (2) studying the mechanism of neutralization in resistant animals, (3) determining the number of hemorrhagins found in various snake venoms, and (4) following purification of hemorrhagins in snake venoms. Further studies will be required to determine if the binding mechanism between the hemorrhagins and antihemorrhagins in the five step western blot and neutralizing activity in the opossum sera are due to the same molecules.

P-081Tu

IMPROVED ELISA FOR RUSSELL'S VIPER *(DABOIA RUSSELII)* ANTIVENOM POTENCY.

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An improved ELISA to assay Russell's Viper (*Daoboia russelii*) antivenom potency was developed by selection of a suitable antigen fraction from the snake's complex venom. Venom was chromatographed on Sephadex G-100 and gave two major peaks (R#1 and R#2), with apparent molecular weights of 170,000 and 21,000 respectively. Using the viper crude venom, R#1 and R#2 as antigens, ELISA's were developed to quantitate the respective horse antibody in 15 batches of antivenom against the viper. The *in vivo* neutralizing activities of these of antivenoms were also determined in mice. Regression analysis of *in vivo* neutralizing activities versus ELISA titers of these antivenoms gave correlation coefficients r of 0.67 ($p < 0.001$), 0.82 ($p < 0.001$) and 0.13 ($p > 0.05$) for the crude viper venom, for R#1 and for R#2, respectively. Thus, for the *in vitro* assay, the high molecular weight venom protein fraction, R#1, served as a better ELISA antigen than the crude venom. This ELISA should be useful to the improvement and standardization of antivenom potency.

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J. Natural Toxins (accepted).

P-082Tu

INHIBITION BY $CaNa_2EDTA$ OF LOCAL TISSUE DAMAGE INDUCED BY *Bothrops asper* VENOM: APPLICATION IN HORSE IMMUNIZATION FOR ANTIVENOM PRODUCTION

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The ability of the chelating agent $CaNa_2EDTA$ to inhibit local tissue damage induced by *Bothrops asper* venom was studied in mice and in horses used for polyvalent (Crotalinae) antivenom production. $CaNa_2EDTA$ was devoid of toxicity when injected i.m. or s.c., inducing only a mild edema. Preincubation of *B. asper* venom with $CaNa_2EDTA$ inhibited hemorrhagic and dermonecrotic activities, but did not reduce edema-forming and myotoxic effects. A group of horses initially immunized with native venoms developed less severe local tissue reactions when injected with booster doses of venom and $CaNa_2EDTA$ than when receiving booster injections of venom alone, although they showed a similar antivenom response. Moreover, antivenoms produced from plasmas of horses that received booster injections of either venom alone or venom plus $CaNa_2EDTA$ had similar neutralizing activity against lethal, hemorrhagic and coagulant effects induced by *B. asper* venom. The similar antibody response was corroborated by Western blotting using crude venom and by an ELISA that estimates anti-myotoxin titer. It is concluded that the chelating agent $CaNa_2EDTA$ reduces the extent of local tissue damage induced by *B. asper* venom, without affecting the immune response of horses against pharmacologically-relevant venom components.

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P-083Tu

DEVELOPMENT OF AN ELISA TO ASSESS THE POTENCY OF HORSE THERAPEUTIC POLYVALENT ANTIBOTHROPIC ANTIVENOM.

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Serumtherapic institutions use large numbers of animals to determine many biological activities of their products, including antivenom potency. Such procedures besides the ethical considerations are costly, demanding stringent protocols to diminish inter-assay variability. An "in vitro" assay would overcome these difficulties and allow some improvements in the determination of potency and purity of antivenoms. We showed (Barbosa *et al.*, (1995) Brz. J. Med. Biol. Res. 1077-1080) a significant correlation of r=0,97 between anticrotalic venom serum Elisa titres and "in vivo" potency, whereas for the antibothropic antivenom a significant correlation could only be obtained with part of the sampled data. To improve this result we tried to isolate a more defined antigen from the *B. jararaca* venom which is officially used for the potency determination of the antibothropic serum. The venom was fractionated in a DEAE-Sephacel column and the fractions with higher correlation between Elisa titres and potency were further fractionated in a Sephadex G-75 column and again tested. Two fractions 1.1 and 1.2 containing haemorrhagic activity displayed r=0,77 and r=0,8 respectively against individual horse sera and r=0,79 and r=0,8 respectively for the ampouled antivenom. For all results p<0,001. Two other fractions with phospholipase activity 4.2 and 4.3 showed r=0,66 (p<0.1) and r=0,56 (p<0.3) respectively against the individual horse antivenom sera. SDS-PAGE control throughout the fractionation process showed an increased purification of the venom fractions, although none reached the level of a single component. The results indicate that despite the multifactorial nature of the lethality of bothropic venom and the polyvalent nature of the antivenom, a significant correlation can be obtained against a more defined toxin.

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P-084Tu

TISSUE AND SERUM KINETCS OF *TITYUS SERRULATUS SCORPION ANTIVENOM*. AN EXPERIMENTAL STUDY IN MICE.

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This work describes the distribution kinetics of the antivenom (IgG Fab'_2) to the venom of *T. serrulatus* scorpion in various tissues and in the serum of CF1 mice intravenously injected with 20 µl of scorpion antivenom diluted in 50µl PBS. A total of 44 animals were divided into groups of four and sacrificed at different time intervals (10 min to 72 h). Blood samples and tissue samples from several organs (heart, lung, liver, kidney, spleen, muscle and brain) were collected. ELISA technique was used for antivenom dosing. Peak antivenom levels in the serum, lung and heart occurred 15 min after injection, and at 1 h, in the liver and kidney. No antivenom was detected in the brain, and antivenom levels in the spleen and muscle were very low. During the first two hours after injection, antivenom concentration decreased rapidly in the lung and liver and more slowly in the serum and other organs, until very low levels were reached at 72 h.

Our study provides basic elements for a better understanding of the biological phenomena resulting from anti-scorpion serotherapy, and can offer subsidies for a more objective treatment for scorpion stings, with a more economical, efficient and controled use of scorpion antivenom in humans.

P-085Tu

NEUTRALIZING ABILITY OF A NEW MONOVALEMT ANTI-*LOXOSCELES INTERMEDIA* ANTIVENOM. COMPARISON WITH ANTI-ARACNIDIC ANTIVENOM

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Loxosceles spider bites have become an increasing public health problem in Brazil. Curitiba is the capital of Paraná State, Southern of Brazil, and for unknown reasons has registered an increasing incidence of bites by the spider *Loxosceles intermedia*. Health Offices have reported more than 3000 cases per year.Two horse-derived antivenoms were tested for the neutralization of lethal and dermonecrotic effects induced by the venom of *Loxosceles intermedia* spider. The antivenoms used were (a) Polivalent anti-Aracnidic antivenom produced by the Instituto Butantan (São Paulo, Brasil). and (b) a new monovalent anti-Loxoscelic antivenom produced with the venom of *L. intermedia* by the Centro de Produção e Pesquisas de Imunobiológicos, Piraquara, Brasil.The antigen for producing the anti-Aracnidic venom plasmas was formed by venoms from: *Tityus serrulatus* and *T. bahiensis* scorpions, *Phoneutria nigriventer* and *Loxosceles gaucho* spiders.The two antivenoms neutralized all the toxic activities tested albeit with different potencies. The new monovalent anti-*L. intermedia* antivenom showed the highest neutralizing ability, suggesting that it would be of high value in the treatment of *Loxosceles intermedia* spider envenomations.
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P-086Tu

POTENCY OF HORSE ANTIVENOMS AGAINST *Bothrops sp* VENOM. CORRELATION BETWEEN *IN VITRO* AND *IN VIVO* ASSAYS

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The usual method for the estimation of antibothropic anti-venom potency involves an *in vivo* neutralization assay using mice. These biological assays gives results which are variable and difficult to reproduce. In the present work an ELISA has been developed for the quantification of antibodies against *Bothrops jararaca* venom (BjV). The correlation coefficients (r) between levels of antibodies measured by ELISA, neutralization of the indirect hemolitic activity and *in vivo* assays were investigated. Thirty horses were hyperimmunized with *Bothrops* venoms (*B. jararaca* 50%; *B. jararacussu* , *B. neuwiedii*, *B. moojeni* and *B. alternatus* 12,5% of each). All antivenoms analyzed effectively neutralized the lethal activity (ED_{50}) at the range of 1.6 to 10.5 mg/ml. The ability of the antibothropic seras to neutralize indirect hemolytic activity of BjV was determined according to Gutiérrez *et al.*, 1989. For the indirect ELISA crude BjV and the toxic fraction (purified from BjV by Sephadex G-100 chromatography) were used as antigens from coating the microtiter plates. When BjV and the toxic fraction were used as antigens in the ELISAs, the values between the ELISA antibodies titers and *in vivo* neutralization of anivenoms were $r = 0.45$ ($P<0.05$) and $r = 0.60$ ($P<0.001$), respectively. When neutralization of indirect hemolysis was correlated with neutralization of lethal activity and ELISA were observed the values $r = 0.46$ ($P<0.02$) and $r = 0.59$ ($P<0.001$), respectively . Thus, the ELISA which measures only the antibody against the principal toxic fraction of the *B. jararaca* venom (reference venom for assesing the bothropic antivenom potency in Brazil) should be most suitable to be used as an in vitro assay for antivenom potency.

P-087Tu

USE OF DIFFERENT IMMUNOGENS FOR PRODUCTION OF HORSE ANTI-SCORPIONIC ANTIVENOM

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In Brazil scorpion envenomation represents a real health problem. Administration of specific anti-venom horse serum is a recognized therapeutic means to circunvent poisoning. Scorpion venom antisera were first produced by immunization with extract of ground venom gland or with crude venom. The present work show the preparation of neutralizing antisera produced by the immunization of horses with three different fractions from *Tityus serrulatus* (Ts) venom. The immunogen preparations used were: (a) Crude venom, (b) Toxic fraction ($TstFG_{50}$ - fraction that represents most of the toxicity of the crude venom after Sephadex G-50 gel filtration) and (c) A non-toxic protein (TsNTxP) purified from the toxic fraction by ion exchange and reverse phase HPLC chromatographic steps. Each horse received an initial injection of 5 mg of soluble Ts whole venom, 2 mg of $TstFG_{50}$ or 0.2 mg of TsNTxP subcutaneously in complete Freund's adjuvant (day 1). Booster injections were made subcutaneously 7, 14 and 21 days later with the same doses emulsified in incomplete Freund's adjuvant. The horses were bled one week after the last injection. The three antivenoms neutralized the toxic effects of the *T. serrulatus* crude venom. Quantities of crude venom varied between to 30 and 50 LD_{50} were effectively neutralized by 1 ml of the each serum. Neutralizing titles were estimated by i.p. injection of CF1 mice. Anti-$TstFG_{50}$ and anti-NTtxP antivenoms showed the highest neutralizing ability. This result shows that the toxic fraction and non toxic protein from *T. serrulatus* venom may be of interest in the production of antivenoms for clinical use.

P-088Tu

Antivenenum and Enzyme -Immuno-Assay
for Cobra venom poisoning treatment

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A project, conducted since 1992, has been completed with the production of an *antivenenum* for treatment of *Naja naja kaouthia* and *Naja naja atra* venom poisoning.

Using the horse antisera produced, an enzyme-immuno-assay has been carried out as following: *Naja naja kaouthia* and *Naja naja atra* venoms were immobilized on CNBr-activated Sepharose 4B. IgG fraction was submitted for specific antibody isolation. Fab and Fab'-s-biotin were then obtained from the immuno-affinity purified antibodies. Polystyrene microplates were coated covalently by a titrated concentration of Fab. Fab'-s-biotin was used as detecting antibody in association with a streptavidin-enzyme conjugated.

Calibration curves obtained by using separately *Naja naja kaouthia* and *Naja naja atra* venoms manifested a same slope from 1 ng/mL to 1 µg/mL of venom concentrations. Probably the high proportions of neurotoxins and cardiotoxins and their homology in both of these venoms could explain the results observed. The sensitivity of the system was 0.1 ng/mL, suggesting a high affinity for the antibodies used. This EIA system will certainly satisfy the need of poisoning evaluation for adequate treatment. Up to now, 36 victims of cobra snake bites were rescued at Cho Ray hospital with the *antivenenum* produced.

P-089Tu

EFFECTS OF ANTICONVULSANT DRUGS ON THE EXPERIMENTAL SEVERE SCORPIONISM ELICITED BY *Tityus serrulatus* VENOM IN MICE.

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Diazepam (DZP) and phenobarbital (PHB) have been used as therapeutic adjuvant to treat seizures in human scorpionism. The aim of this study was to analyze the effects of DZP, PHB and phenytoin (PHT) on the convulsions induced by the crude lyophilized ***Tityus serrulatus*** scorpion venom (1.5 mg/kg). Each experimental group consisted minimum of 12 animals and all drugs were injected intraperitoneally. The percentage of animals that developed convulsions, the time latency to the first convulsion and the number of convulsive crises were determinated. DZP (0.3, 1.0, 3.0 and 10 mg/kg) did not modify the percentage of animals that progressed to convulsions when compared to control (86.2%). DZP (0.3 and 1.0 mg/kg) had no effect on the time latency to the first convulsion whereas the higher doses used (3.0 and 10.0 mg/kg) increased the convulsive thresholds (35.4 ± 4.4 min, $P<0.001$ and 36.5 ± 5.8, $P<0.005$; respectively) when compared with threshold values in controls (20.4 ± 1.6 min). Only the higher doses of DZP (3.0 and 10.0 mg/kg) decreased the number of convulsive crises (4.3 ± 0.9, $P<0.05$ and 2.5 ± 0.5, $P<0.01$; respectively) when compared to controls (8.6 ± 1.2). PHB (3.5, 10.0 and 30.0 mg/kg) did not modify both the percentage of animals that developed convulsions and the latency time to the first convulsion. PHT (3.5 and 10.0 mg/kg) did not alter the frequency of convulsions but the highest dose (30.0 mg/kg) increased the number of crises (13.4 ± 2.1, $P<0.001$). PHT (3.5, 10.0 and 30.0 mg/kg) decreased the percentage of mice that developed convulsions (58.3%, $P<0.05$; 66.7%, $P<0.05$ and 75.0%, $P<0.05$; respectively). PHT in all doses tested neither modified the convulsive threshold nor the number of crises in those mice that progressed to seizures. In conclusion, both DZP and PHT have differential protective effects on venom-induced seizures whereas PHB can increase the severity of the convulsive phenomenon.

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P-090Tu

PHENYTOIN PROTECTS AGAINST THE LETHALITY INDUCED BY THE *Tityus serrulatus* SCORPION VENOM IN MICE.

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It is thought that the toxicity of ***Tityus serrulatus*** scorpion venom is mainly due to toxins active on voltage-gated Na^+ channels of excitable cells. Tityus toxins affect the process of channel inactivation with a subsequent increase in the Na^+membrane permeability. Phenytoin is a well known antiseizure agent widely used in the therapy of epilepsies. Phenytoin also interacts with voltage-gated Na^+ channels but, unlike the scorpion venom, reduces Na^+ currents. The present study analyzes the effects of phenytoin on the 24h-lethality induced by the intraperitoneal administration of crude lyophilized scorpion venom, 1.5 mg/kg, in mice. The effects on lethality of specific scorpion antivenom were also investigated in the same experimental protocol. Pretreatment with phenytoin, 1.0 mg/kg *ip*, did not modify the venom-induced lethality (58.3%, n=12) when compared to the corresponding control (72.2%, n=18). In contrast, phenytoin, 3.5 mg/kg *ip*, markedly decreases the lethality to 33.3% ($P<0.05$, n=12). The highest dose of phenytoin, 10 mg/kg *ip*, also reduced the lethality to 33.3% ($P<0.05$, n=12). When animals were pretreated with antivenom, 0.5 ml/kg *ip*, the lethality induced by the venom (60.7%, n=28) did not differ from that observed in the corresponding control (76.9%, n=52). Higher doses of antivenom, 2.0 ml/kg *ip* and 12.5 ml/kg *ip*, decreased lethality to 34.8% (n=23, $P<0.001$) and 28% (n=25, $P<0.001$), respectively. Present results suggest that phenytoin might be an alternative approach for the treatment of intoxication with ***Tityus serrulatus*** scorpion venom. The protection of phenytoin against the lethality induced by ***Tityus serrulatus*** venom could be a result of the antagonistic effects of both agents on Na^+ channels.

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P-091Tu

TH1 AND TH2 FUNCTIONS IS DOWN REGULATED BY *Crotalus durissus terrificus* VENOM

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We have previously demonstrated the supressive effect of *Crotalus durissus terrificus* (cdt) venom on the *in vivo* TH2-dependent humoral immune response. Mice pre-treated with cdt venom before administration of human serum albumin (HSA) showed significant reduction of IgG1 anti-HSA antibody levels (TOXICON:35, 607-612,1997). Here we report that this TH2 down regulation of the antibody response can be transferred by spleen cells. The production of IgG1 anti-HSA antibody was decreased when non-treated mice received, before HSA immunization, purified T-cells obtained from the spleen of donors which were pre-treated with cdt venom.
The suppressive effect of cdt venom on the *in vitro* proliferative response and the TH1 and TH2 cytokine production induced by ConA stimulation is also reported. Spleen cells from Balb/c mice were either pretreated with cdt venom or treated with cdt venom simultaneously with ConA stimulation (co-cultivation). The possible cytotoxic effect of cdt venom was also examined. The spleen cell proliferation evaluated by MTT colorimetric assay showed an inhibitory effect of cdt venom on proliferation induced by ConA but no cytotoxicity effect. The inhibition was as more intense as earlier the cdt venom treatment was initiated. The level of IL-2, INF-γ, IL-4 and IL-10 cytokines produced 24 and 48 hr after ConA stimulation was evaluated by ELISA test. We observed that the level of all cytokines studied was decreased and the pretreatment was more efficient than the co-cultivation in inhibiting the cytokine production. Taken together our data suggest that cdt venom interferes with the normal function of TH1 and TH2 populations with consequent inhibition of the cellular *in vivo* and *in vitro* immune response.
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P-092Tu

DEVELOPMENT OF SNAKE ANTIVENOM IN CHICKEN AND ITS RECOVERY FROM THE YOLK AS THERAPEUTIC TOOL FOR DOMESTIC ANIMALS

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Adult Leghorn white hens hyperimmunized with brazilian snake venoms, genera *Bothrops*, *Crotalus* or both, produced antibodies capable of recognizing, combining and neutralizing their toxic and lethal components. The antibodies, screened by the enzyme-linked immunosorbent assay (ELISA) were first detected two weeks after starting the immunization schedule, attaining the highest titers by third week and remaining high until at least 168 days of observation. These antibodies are transfered to the egg yolk from where can be isolated as enriched IgY preparations through combination of methods using positive and negative precipitation with sodium sulphate and/or caprilic acid. The yolk derived IgY preparations, obtained with acceptable specific activity, recovery and yield, contained antibodies endowed with the ability of blocking the phospholipase A2-dependent hemolysis activity of both venoms and the hemorrhagic activity of Bothrops venom, and of neutralizing the toxic lethal activity of Bothrops venom (ED50 = 592.5 µL /2DL50 and potency = 0.0675 mg/mL) of Crotalus venom (ED50 = 457.5 µL/3DL50 and potency = 0.075 mg/mL).
Supported by FENORTE and CNPq

P-093Tu

KINETIC ANALYSIS OF PORE FORMATION BY STICHOLYSIN I.

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Potent cytolysins have been extracted from the venom of at least 16 species of sea anemones. At subnanomolar concentration they cause erythrocyte lysis by opening pores, probably oligomeric. Consequently they have received the family name of actinoporins. Sticholysin I (St I) is an actinoporin isolated from the venom of the Caribbean sea anemone Stichodactyla helianthus. To elucidate the molecular mechanism of pore formation, we have studied the kinetics of interaction with lipid membranes. The time course of permeabilisation was detected by measuring calcein release from uniform-sized large unilamellar vesicles. Channels features were determined using voltage-clamped planar lipid bilayers and partition coefficients were estimated by centrifugation and electrophoresis. Under optimal conditions: presence of 50% sphingomyelin (SM), pH around 8 and St I/vesicle ratio <200, most of the toxin is bound to the lipid phase. St I effect was reduced at high or low pH and high SM content, mainly due to decreased binding. From the dose-dependence it was inferred that St I forms oligomeric pores comprising at least 3 monomers. The kinetics of release was fully accounted by a model assuming that trimerisation leads to a conductive pore. All the relevant equilibrium and rate constants were derived. Addition of St I to one side of planar lipid membranes increased the conductivity of the film in discrete steps of defined amplitude, indicating the formation of ion channels. Pores were cation-selective and exhibited rectification and voltage-dependent gating. The channel conductance suggested a functional radius of about 1.0 nm.

P-094Tu

Production and Characterization of Neutralizing Antibody Against a Thrombin-simile Purified from *Bothrops atrox* Venom.

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Snake venom contains a complex mixture of proteins and pharmacologically active peptides. The most relevant symptoms em *B. atrox* envenomation is alterations of the blood coagulation pattern. A thrombin-simile protease was purified from *B. atrox* by a combination of gel exclusion and ionic exchange chromatography. The enzyme is a 35 kD protein with a N-terminal sequence VIMXDEXDINEXPFLAFMYYPQYF, presenting 80% of homology with a thrombin-simile previously described for *B. moogeni* . We developed a panel of monoclonal antibodies (Mabs) obtained from BALB/c mice immunized with denatured and non-denatured purified enzyme . Mabs produced by spleen cells from mice immunized with non-denatured enzyme were very effective to abolish the fibrinogen coagulating activity of a reference thrombin-simile enzyme, whereas Mabs produced by cells from mice immunized with denatured enzyme were only partially effective to block the enzyme. These differences may suggest that the original molecular conformational of the enzyme retains its active site to be properly recognized and presented by the immune competent cells.
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P-095Tu

DESIGN, SYNTHESIS AND FUNCTIONAL EXPRESSION OF RECOMBINANT Pi1, A K^+-CHANNEL BLOCKER

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Pi1 was the first K^+-channel blocking peptide described to contain 4 disulfide bridges, instead of three normally found in this class of scorpion toxins (Olamendi-Portugal, T. *et al, Biochem. J.* 315:977-981, 1996). A synthetic gene coding for Pi1 was prepared from two oligonucleotides, by the recursive PCR method, and expressed in *E. coli*. The fusion protein containing Pi1 was liberated from the hybrid by tryptic digestion and purified by HPLC. The correct amino acid sequence and composition of the recombinant peptide (rPi1) were confirmed by automatic Edman degradation and by amino acid analysis. The capacity of rPi1 to block K^+-channels was verified by patch-clamp experiments using Sf9 cells infected with a baculovirus containing the messager that codes for the *Shaker*B K^+-channel.

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P-096Tu

EPITOPE MAPPING FOR MONOCLONAL ANTIBODIES AGAINST LTX

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It was shown previously in the experiments on synaptosomes that some monoclonal antibodies (mAbs) against α-Latrotoxin (LTX) from the black widow spider venom inhibited functional activities of the toxin crucially. These mAbs were chosen to find the sites on the LTX molecule responsible for the execution of LTX effects: mAb A24 blocked both Ca^{+2} uptake and neurotransmitter release completely, mAb A4 prevented Ca^{+2} influx completely but only partially secretagogue activity of LTX, mAb YAL7 decreased the fusogenic effect of the toxin.
Three approaches were used in the study: toxin hydrolysis and identification of immunoreactive peptides, expression of different LTX gene fragments in *E.coli* and analysis of their interaction with mAbs, screening of the synthetic overlapping peptides library. At the first step the smallest BrCN peptides recognized by the mAbs were sequenced and the high immunogenic region on the LTX molecule was shown to contain the antigenic determinants of all three mAbs. To confirm the obtained data several fragments of the LTX gene were cloned in pT7-7 and expressed in *E.coli*. A4 reacted with the fragment Met^{1}-Asn^{341}. Taking into account the peptide sequence data the A4 epitote seemed to lie within the residues Phe^{234}- Met^{314}. The fragment Asn^{341}- Asp^{360} reacted with mAbs A24 and YAL7. Immunochemical analysis of the overlapping synthetic peptides covering the latter fragment showed that A24 recognize the sequence ^{344}QISFDK^{349} and YAL7 is certain to react with ^{350}DITTPI^{355}. The obtained results clarify understanding of LTX functional domain localization and can serve as a basis in the further investigation of neurosecretion processes.

P-097Tu

OsK1 cDNA CLONING AND EXPRESSION IN *E.coli*

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Toxin OsK1 isolated from the venom of scorpion *Orthrochirus scrobiculosus* was shown to block small-conductance Ca^{2+}-activated K^+-channels in NG 108-15 cells. The DNA sequence encoding OsK1 was constructed from mRNA isolated from the scorpion venom glands using Rapid Amplification of cDNA Ends (RACE) polymerase chain reaction. No difference was observed between the amino acid sequence deduced from cDNA and that determined by protein chemistry methods. Because of difficulties in expressing of small proteins enriched with disulfide bonds in bacteria directly a fusion protein strategy was adopted. A recombinant expression plasmid was obtained by insertion of DNA fragment containing the toxin coding region into the expression vector PEZZ18. The recombinant toxin was expressed in *E.coli* HB101 as a fusion protein with two IgG-binding (ZZ) domains of protein A from *Staphylococcus aureus*. The soluble hybrid protein was purified up to 80% purity in the milligram range by a single immuno-affinity chromatography on IgG-Sepharose. Recombinant OsK1 was obtained from fusion protein by trypsin cleavage and finally purified by reversed phase HPLC. The composition of the purified toxin was verified by mass spectrometry and N-terminal amino acid sequencing. The toxicity of hybrid protein and recombinant OsK1 was tested *in vivo*. It was demonstrated that the biological activity both of fusion protein and recombinant OsK1 was comparable to the activity of native toxin.

P-098Tu

FUNCTIONAL EXPRESSION OF δ-LATROINSECTOTOXIN IN *E.COLI* CELLS.

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The black widow spider venom contains a family of high-molecular-weight neurotoxins that stimulate secretion of various neurotransmitters in vertebrates and invertebrates. Despite a large body of data obtained by methods of biochemistry, electrophysiology and molecular biology the latrotoxins molecular mechanism of action is unclear yet. More detailed studies of this problem are limited by the absence of methods allowing expression of latrotoxins and their fragments in active forms. The structural gene of δ-latroinsectotoxin (δ-LIT) was earlier expressed using the system based on T7 RNA polymerase in *E. coli* BL21 (DE3) cells. The recombinant δ-LIT was mainly synthesized as insoluble inclusion bodies. The goal of this work was the development of a method for purification and refolding of δ-LIT to obtain its functionally active form. The inclusion bodies were isolated and dissolved in a buffer containing 8 M urea, and inactive δ-LIT was purified by ion-exchange FPLC. After purification, the toxin was treated with dithiothreitol to reduce intermolecular disulfide bonds. The optimal dithiothreitol concentration and renaturation conditions were selected. To renaturate δ-LIT the concentration of denaturing agents in the toxin preparation was decreased tenfold, and the toxin was oxidized on the air for 48 h. After concentrating, the toxicity of the recombinant δ-LIT was assayed in *Musca domestica* larvae. The developed procedure allowed us to obtain functionally active recombinant toxin with toxicity comparable to that of native δ-LIT. The yield of the recombinant δ-LIT was 0.2-0.3 mg/l culture.

P-099Tu

CLONING OF GENES ENCODING ISOFORMS OF α-NEUROTOXINS IN *NAJA NAJA SPUTATRIX* VENOM

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Malayan cobra (*Naja naja sputatrix*) venom contains post-synaptic neurotoxins that prevent the binding of acetylcholine to its receptors. By RT-PCR and Advantage™ genomic-PCR we obtained information for the presence of four isoforms of short neurotoxins in the venom of *N.n.sputatrix*. The cDNAs obtained by RT-PCR were subcloned and expressed in *E.coli*. The recombinant proteins were characterized both by western blotting and N-terminal protein sequencing. Our Advantage™ genomic-PCR results so far confirm the presence of at least 3 short neurotoxin genes in the genome of *N.n.sputatrix*.

P-100Tu

GENES ENCODING ISOFORMS OF CARDIOTOXIN IN *NAJA NAJA SPUTATRIX*

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The venom of cobras and Ringhals are unique from the other members of the *Elapidae* family snakes, in having cardiotoxins. The venom of *Naja naja sputatrix* (Malayan spitting cobra) is rich in cardiotoxins and account for about 60% of the venom dry weight. Cardiotoxins are basic polypeptides containing 60-63 amino acid residues in a single polypeptide chain, crossed-linked by four disulfide bonds. Using the method of reverse transcription-polymerase chain reaction (RT-PCR), we have identified 9 cDNA sequences encoding 6 isoforms of cardiotoxins in *Naja naja sputatrix*. Following expression in *E. coli*, the recombinant proteins were confirmed to be cardiotoxin by western blotting, N-terminal sequencing, as well as their cytolytic activity on cultured HeLa cells. The origin of these isoforms were traced to 6 different genes on the genomic DNA obtained from a single Malayan spitting cobra by genomic-polymerase chain reaction. To our knowledge, this is the first report of the gene structure of cardiotoxin from a snake venom.

P-101Tu

GENOMIC ORGANIZATION OF A K⁺-CHANNEL TOXIN GENE FROM SEA ANEMONE : A COMMON PATH OF EVOLUTION AMONG ION-CHANNEL TOXIN GENES ?

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Sea anemones have attracted substantial attention recently as host to the first K⁺-channel toxins to be isolated from a marine animal (1,2,3). These toxins represent a new structural class of K⁺-channel-blocking peptides. The ability of one of these molecules to potently inhibit the Kv 1.3 potassium channels in lymphocytes (4) has made these toxins potential candidates as immunosuppressants. This led to a near complete structural and functional characterization of these molecules. We have recently also provided the first cloning and functional expression data for a new K⁺-channel toxin, HmK, from the tropical sea anemone, *Heteractis magnifica*, thus providing an understanding of the regulation of biosynthesis and processing of these molecules (5). In this communication, we provide information on the genomic organization of *H. magnifica* K⁺-channel toxin gene. A series of DNA amplification by PCR which included amplification of 5'-UTR was used to map intron/exon junctions in the HmK gene. In the first amplification, the DNA fragments obtained from PCR of genomic DNA, and that of control RT-PCR reaction performed on total *H. magnifica* RNA, using a set of primers specific to HmK gene were of the same length and sequence, implying that the coding region of matured HmK toxin is intronless. In a second amplification, using two primers, a sense primer that annealed to the beginning of the leader sequence and an antisense gene-specific primer, followed by sequence analysis showed that a single intron of 402 nucleotides separated the leader sequence from the sequence encoding matured toxin. Two possible transcription-initiation sites were identified by primer extension analysis. Corresponding TATA box consensus sequences, characteristic of promoter region, were also located from PCR products of uncloned libraries of adaptor-ligated genomic DNA fragments. The coding region for matured HmK is intronless. The same is also true for other sea anemone toxins reported thus far. More notably, similar intron-exon organization is present in other ion-channel blocking toxins from scorpions implying that molecules having similar functions share similar organization at genomic level suggesting a common path of evolution.

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P-102Tu

Cloning and sequence analysis of a *Bothrops jararaca* cDNA encoding a precursor of seven bradykinin-potentiating peptides and a C-type natriuretic pepitde

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A 1.8-kb cDNA clone was isolated from a *Bothrops jararaca* venom gland cDNA library that encodes a 256-aa precursor for bradykinin-potentiating peptides (BPP) (angiotensin-converting enzyme inhibitors) and a C-type natriuretic peptide (CNP). The seven bradykinin-potentiating peptides are aligned tandemly after the hydrophobic signal peptide sequence, followed by a putative intervening sequences and a CNP at the C terminus. Northern blot analysis indicated the predominant expression of a 1.8-kb mRNA in the venom glands as well as in the spleen and the brain. Two lower intensity mRNA bands of 3.5 kb and 5.7 kb also hybridized to the cDNA clone. Radioimmunoassay for the CNP was performed using the antiserum against rat CNP. The presence of CNP immunoactivity was detected in the low molecular weight fraction of the *Bothrops jararaca* venom.

P-103Tu

Cloning and sequence analysis of serine protease cDNAs from *Bothrops jararaca*

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Snake venoms of the crotalinae species contain an abundance of serine proteases. We isolated cDNA clones encoding serine protease from *Bothrops jararaca* venom gland cDNA library. They contain cDNA insert of 1.3-3.8 kbp. The differences in length are mainly due to insertions/deletions of 3'-untranslated region and differences in positions of poly A addition. They encode preproprotein of 253-258 amino acid residues. Catalytic triads common to serine proteases (His-41, Asp-91, Ser-178 of bothrombin) are conserved except that protein for a clone HS#120 contains glutamine instead of His-41. The position of 12 half-cystines are identical among all the sequences, suggesting that the proteins encoded will take tertiary structures closely related each other. Comparisons of nucleotide sequences indicate that the nucleotide substitutions in the coding region have occurred more frequently than in the untranslated regions and that nonsynonymous substitutions have occurred more frequently than synonymous substitutions. These findings in serine protease cDNAs of *B. jararaca* are consistent with the concept of accelerated evolution reported for serine protease and phospholipase A_2 from *Trimeresurus* genus venom gland.

P-104Tu

CLONING, EXPRESSION AND SEQUENCE ANALYSIS OF A NEW METALLOPROTEINASE/DISINTEGRIN FROM *Agkistrodon contortrix laticinctus*

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Disintegrins comprise a family of RGD-containing peptides that are potent inhibitors of platelet aggregation and cellular adhesion. Large hemorrhagic metalloproteinases from snake venoms also have a disintegrin domain although they lack the RGD sequence. Precursor forms of disintegrins, large and short snake venom metalloproteinases (svMP) share high degree of similarity on both cDNA and amino acid sequences. These proteins are of great interest due to their potential application in thrombosis and adhesion research. From an *A. c. laticinctus* venom gland cDNA library made using the ZAP® vector, we have isolated and sequenced a cDNA (ACLPRED) encoding a new metalloproteinase with a disintegrin domain. ACLPRED is a 2407 bp in length and it has an open reading frame of 1863 bp, predicting a pro-enzyme form of 621 amino acids and a mature protein of 432 residues (ACLD). The cDNA sequence has the highly conserved 5'and 3' untranslated sequences, as well as the also conserved pro-enzyme region. The predicted sequence for the metalloproteinase domain has the conserved zinc binding motif, followed by the non RGD-disintegrin and cysteine-rich domains. Sequence comparison of precursor forms of RGD-disintegrins, short svMP, and metalloproteinase-disintegrin proteins reveals that the precursor forms of RGD-disintegrins share higher similarity with the short svMP than the metalloproteinase/disintegrin group due to the presence of some very conserved amino acid residues in the processed groups, suggesting a role for those residues in the processing of the disintegrin domain. The cDNA sequence coding for the mature protein was amplified by PCR and subcloned into a pET28® vector for expression in BL21(DE_3) *E. coli* cells, leading to an expression of a 55 kDa protein.

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P-105Tu

PRESENCE OF MYOTOXIN *a* mRNA IN VENOM GLAND AND LIVER SAMPLES FROM PRAIRIE RATTLESNAKES (*Crotalus viridis viridis*)

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Myotoxin *a*,a small, basic polypeptide found in the venom of the Prairie Rattlesnake (*Crotalus viridis viridis*) induces necrosis of skeletal muscle cells after injection into mice. Although responsible for part of the local tissue damage resulting from bites by these snakes, it is not effectively neutralized by commercial antivenom.SDS-PAGE analysis of venoms from 13 Prairie Rattlesnakes collected from the same den site showed that 5 of the venoms contained myotoxin *a* whereas 8 venoms lacked the toxin. This pattern was consistent over a three year period. We developed a hypothesis that although all of the Prairie Rattlesnakes have the gene for myotoxin *a*, only the snakes which produce the toxin actually transcribe the gene and thus have the mRNA for myotoxin *a*. To test this hypothesis, we used both RT-PCR and *in situ* hybridization techniques. Venom glands, liver, pancreas and spleen were removed four days after extraction of venom from one positive and one negative snake. Total RNA was extracted from one venom gland and part of the liver from each snake while the other venom gland, part of the liver and other tissues were fixed in formalin, processed, embedded in paraffin and sectioned for microscopy. Using two non-degenerate primers, RT-PCR of venom glands and livers resulted in a product of about 200bp, the expected size for a cDNA from myotoxin *a* mRNA. The sequence of the RT-PCR product from each venom gland matched the sequence for myotoxin *a* cDNA previously published. Thus, mRNA for myotoxin *a* appears to be present in venom glands and livers from both myotoxin *a*-producing snakes as well as non-producing snakes. *In situ* hybridization studies are in progress.

P-106Tu

CLONING AND SEQUENCE ANALYSIS OF GENES ENCODING PHOSPHOLIPASE A_2 IN THE VENOM OF *NAJA NAJA SPUTATRIX*.

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Snake venom phospholipases A_2 (PLA_2) are hydrolytic enzymes with diverse pharmacological activities. Their mechanism of action is still uncertain. PLA_2, forms one of the most toxic component (10%) of the *Naja naja sputatrix* venom. cDNAs encoding two isoforms of neutral phospholipase A_2 and one acidic PLA_2 from *N.n.sputatrix* venom gland have been cloned and characterized by RT-PCR. In this report we present evidence for the presence of genes encoding these group 1 PLA_2, in the same genome of *N.n. sputatrix*. The sequences and the structures of these genes as well as their phylogenetic relationships with their counterparts are discussed.

P-107Tu

LONG AND WEAK NEUROTOXINS FROM *NAJA NAJA SPUTATRIX*: cDNA CLONING AND SEQUENCE ANALYSIS.

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Functionally similar post-synaptic neurotoxins from snake venom can be classified into two sub-groups, short (60-62 amino acid residues) and long (70-74 amino acid residues) neurotoxins. Several short and long neurotoxins have been identified and their amino acid sequences have been determined. However, no nucleotide sequence information is available to date, from any of the long neurotoxins. The weak neurotoxins, belonging to the group of short neurotoxins, exhibit a weak or reduced neurotoxicity. In this report we present evidence for the presence of a long neurotoxin and two isoforms of weak neurotoxin in the venom of *Naja naja sputatrix* by cloning and sequence analysis of the corresponding cDNAs obtained by RT-PCR.

P-108Tu

CROTALINAE SNAKE VENOM GLAND SERINE PROTEASES HAVE EVOLVED *VIA* DARWINIAN-TYPE ACCELERATED EVOLUTION

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We have recently shown that crotalinae snake venom gland phospholipase A2 isozyme genes have evolved in an accelerating manner to gain diverse physiological functions of their products. To establish that this is a common phenomenon in crotalinae snake venom gland isozymes, serine proteases in venom gland have been studied. More than ten cDNAs encoding serine proteases have been isolated from *Trimeresurus flavoviridis*, *T. gramineus*, and *T. okinavensis* venom gland cDNA libraries. As well, three genes coding for venom gland serine proteases have been cloned from *T. flavoviridis* liver DNA library. Comparison of their nucleotide sequences indicated that nonsynonymous nucleotide substitutions have accumulated in the mature protein-coding regions to cause amino acid change while the noncoding sequences including introns are highly homologous to one another. Southern blot analysis of *T. flavoviridis* genomic DNAs showed that venom gland serine protease genes form a multigene family. These observations suggest that venom gland serine proteases have diverified their amino acid sequences in an accelerating manner. Thus, accelerated evolution appears to be universal in plural isozyme families of crotalinae snake venom gland.

P-109Tu

Structure of Group I Snake Venom Phospholipase A_2 Gene from *Laticauda semifasciata*

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Secretory phospholipases A_2 (PLA_2)[EC 3.1.1.4] are classified into two major groups (Group I & II), based on their primary structure features. They share six disulfide bonds but the seventh is difficult in two groups. Group I PLA_2s (Elapidae snake venoms and mammalian pancreas) has seven disulfide bonds, while group II PLA_2s (Viperidae snake venoms and mammalian non-pancreatic enzymes) has six disulfide bonds. Phospholipases A_2 in the venom of *Laticauda semifasciata* belong group I. Phospholipase A_2 genes, encoding snake venom, are specifically and abundantly expressed in the venom glands. Snake group II PLA_2 genes have been studied very well. But there are no reports on snake group I PLA_2 gene structure. We have determined the nucleotide sequence of PLA_2 cDNA clones from the library derived from the venom gland of *Laticauda semifasciata*. The specific expression of the gene in the venom gland is confirmed by Northern hybridization analysis. By direct PCR using genomic DNA as a template, we found that the PLA_2 genes are 3.4 and 3.6 kbp in length and the distances between the genes are about 7 kbp. Recently, we have constructed full genomic library from *Laticauda semifasciata* liver. Using the amplified PLA_2 gene fragment as a probe, we have been screened several PLA_2 gene clones from the library. Nucleotide sequences of these clones will be presented.

P-110Tu

Classification of sea snakes in genus *Laticauda* by nucleotide sequences encoding short chain neurotoxins

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The genus *Laticauda* is distributed over a very extensive region of South Pacific area. We used three species of *Laticauda* (*L.laticaudata, L.colubrina* and *L.semifasciata*) from six areas (Okinawa, Tonga, Fiji, Vanuatu, New Caledonia and Philippines) as samples. We attempted to determine nucleotide sequences of cDNAs encoding snake short chain neurotoxins to investigate the relationships between classification by the nucleotide sequence and by the phenotype. Total RNA was extracted from the venom glands. Poly(A)+ RNA was purified by oligo(dT) paramagnetic polystyren beads (Dynabeads oligo$(dT)_{25}$; Dynal, Oslo, Norway). The cDNA fragments were obtained by RT-PCR with the primers for snake neurotoxin gene. The fragments obtained (about 500 bp) were cloned into TA-vectors (Promega, Madison, USA) and sequenced. The sequences obtained (40 sequences from three species) were aligned and compared. Each sequence has mutations specific to species and its habitation area. We also made a phylogenetic tree from the aligned sequences. Genetical classification by cDNA sequences was almost the same as morphological one. We can determine the species and habitation area from the nucleotide sequence of cDNA encoding short chain neurotoxin.

P-111Th

NEUTROPHIL MIGRATION INDUCED IN MOUSE BY THE STAPHYLOCOCCAL ENTEROTOXIN TYPE A: A PHARMACOLOGICAL ANALYSIS. **Ivani A. Desouza** and G. Ribeiro-DaSilva, Department of Pharmacology, FCM, UNICAMP, CP 6111, 13084-100, Campinas, SP, Brazil.**

Staphylococcal enterotoxins (SEs), the most common cause of food poisoning, produce emesis and diarrhea in humans and monkeys but have little or no effect in non-primate animals (1,2). Recently, we demontrated that staphylococcal enterotoxin B (SEB) induces neutrophil migration into the mouse peritoneal cavity and that this phenomenon involves lipoxygenase (LO) metabolites and resident macrophages (3). In the present work, we have investigated the mechanisms of staphylococcal enterotoxin A (SEA)-induced neutrophil migration into the mouse peritoneal cavity. SEA (16-64 µg) diluted in 0.1 ml of PBS was injected ip into Swiss mice (5 mice/group). Control mice received 0.1 ml of PBS alone. The neutrophil migration was measured 4,12, 24 and 72 h after injection of the toxin. SEA induced significant neutrophil migration into the mouse peritoneal cavity (Control: $3 \pm 0.5 \times 10^5$ *vs* SEA: $19 \pm 3 \times 10^5$*/cavity). This migratory effect was dependent on the number of resident macrophages (Normal mice: $9 \pm 1 \times 10^5$ *vs* Thioglycolate-treated mice: $28 \pm 3 \times 10^5$). Dexamethasone (0.5 mg/kg) inhibited SEA (32 µg)-induced neutrophil migration by 40%. A similar response was observed when the mice were pretreated with BWA4C (LO inhibitor, 10 mg/kg); with diphenhydramine (2 mg/kg) or with BN52021 (PAF inhibitor, 10 mg/kg). Neither indomethacin (5 mg/kg) nor cimetidine (2 mg/kg) reduced the chemotactic activity of SEA. Our data indicate that SEA-induced neutrophil migration into the mouse peritoneal cavity is dependent on the number of resident macrophages and involves LO mediators. Since the latter mediators are also responsible for the emesis and pseudo-allergy produced by SE in monkeys, our data suggest that the SEA-mediated phlogistic reaction in mice may be a valuable model for studies employing SE.**1.** Fleischer, *et al., Cell. Immunol.* 119: 92, 1989. **2.** Jett, *et al.,* Infect. Immun. 58: 3494, 1990. **3.** Desouza, *et al., J. Natural Toxins, in press,* 1996. **FINANCIAL SUPPORT: FAPESP (94/0210-2 and 95/0390-3) and FAEP/UNICAMP (0263/95)**

P-112Th

Structural organization of the different domains from Cry1Ab toxin of *Bacillus thuringiensis* in the membrane-inserted state.

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The Cry toxins of *Bacillus thuringiensis* (*Bt*) are widely used as biopesticides because in addition to have toxic activity against specific insects, they are innocuous for other animals and plants. One of the most interesting topics in the *Bt* field is the study of Cry toxins mechanism of action.

Upon ingestion by susceptible insects, the crystals are solubilized and the protoxins are activated by gut proteases. Then, the toxin binds to high affinity receptors on the surface of the midgut epithelial cells and it is thought that there is a change in toxin conformation which is needed for toxin insertion into the lipid bilayer and K^+ channel pore formation. The major effect on intoxicated cells is one over their ionic balance. K^+ and water rapidly enter into the cell and it eventually swell and burst causing a loss of gut integrity and resulting in larval death within few days.

Although it is known the crystal structure of Cry1Aa and Cry3A, at present it is not known neither which are the toxin's conformational changes that are needed to become a insertion-competent state, nor how Cry toxins are inserted in the membrane.

The objective of this work is to analyze, at structural level, the conformation of Cry1Ab toxin when it is inserted into the membrane. In particular, we will make emphasis in the study of the conformational changes among the three different domains of the protein using a fluorescence energy transfer approach.

P-113Th

ISOLATION AND CHARACTERIZATION OF PEPTIDES FROM A PHAGE VARIANT LIBRARY WHICH ARE ABLE OF BINDING TO CRY1A(b) δ-ENDOTOXIN AS ITS NATURAL RECEPTOR

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Bacillus thuringiensis is a Gram-positive, spore forming bacterium characterized by it is ability to produce a proteinaceous parasporal crystal during sporulation. These crystals called δ-endotoxinas or insecticidal crystal proteins (ICP). ICPs exhibit a highly specific insecticidal activity. Two types of proteins are present in the crystals, the Cry and Cyt toxins.

Three domains are present in the Cry toxins domain I, has been proposed as a pore-forming domain, the domain II it is involved in receptor binding in most toxins. Domain III is thought to be important for the stability and selectivity of the toxin.

We are interested in the mecanism of the binding of the toxin CryIA(b) with its receptor. We will try isolate peptides that are capable of binding CryIA(b) δ-endotoxin with it is natural receptor by means of phage display. The peptides come from a single chain variable fragment library (scFv), wich are expresed in M13 phage.

Once the peptides that bind to the toxin are isolated, we will select phages that could bind to loop II (368RRPFNIGI375) of the CryIA(b) toxin by fixing a CryIA(b) mutant afected in loop II (368AAPFNIGI375), that mutant dont bind the receptor. The phages that do not bind to the mutant, are candidates to be those thats interact with loopII of the native toxin.

We will allso try to prove, via phage display, the participation of domain III in CryIA(b) and CryIA(c) toxins determines specificity.

P-114Th

IS BACTERIAL ADHERENCE THROUGH CFA-I A NECESSARY STEP FOR SECRETION OF THE PERIPLASMIC HEAT LABILE TOXIN IN ENTEROTOXIGENIC *Escherichia coli*?

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Enterotoxigenic *E. coli* is a major cause of diarrhoea. To promote colonization of the host intestine, ETEC produces adhesins or colonization factor antigens (CFA), which recognize specific carbohydrate receptors on the intestinal membrane. ETEC also synthesizes enterotoxins; heat stable enterotoxins (ST) are secreted to the extracellular environment, while heat-labile enterotoxins (LT) remain in the periplasmic space. In order for LT to reach its membrane receptor, and cause diarrhoea, it has to be released. We hypothezise that CFA binding to its receptor causes changes in the bacterial membrane that result in LT secretion. Using *in vitro* and animal models, we assayed isogenic *E. coli* strains H10407 and H10407P (CFA-I $^{+}$/LT^{+} and CFA-I^{-}/LT^{+} respectively) to determine whether the colonization factor CFA-I is necessary for LT secretion. For the *in vivo* model, we measured accumulation of intestinal fluid in rabbit ileal loops. LT concentration was determined by GM-1 ELISA. In the *in vitro* system LT was determined by cAMP increase in CaCo2 cells after incubation with the bacterial strains. We found that LT was produced in both cases, and there was no apparent relationship between LT secretion and the presence or absence of CFA-I. However antibodies against CFA-I were sufficient to block the accumulation of intestinal fluid caused by CFA-I $^{+}$/LT^{+} strain in the rabbit ileal loops.

P-115Th

DIFFERENCES IN MODE OF ACTION OF DOMAIN I FROM CRY 4A AND CRY 11A TOXINS FROM *Bacillus thuringiensis* IN MOSQUITO MIDGUT MEMBRANES

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Bacillus thuringiensis are gram-positive soil bacteria. These species produce a wide variety of toxins, active in different insects, namely Cry and Cyt proteins. Cry proteins were classified in relation with its amino acid identity, clustering into 19 families.

Cry toxins are composed of three domains. Domain I have 7 α-helices, the helix α-5 is surrounded by 6 α-helixes. This is the pore-forming domain. Domain II consists of three anti parallel β-sheets and it is implicated in receptor recognition in the insect midgut. Domain III is formed by a β-sandwich. Its function is to stabilize the protein structure and it is also involved in receptor interaction.

A phylogenetic analysis of the primary structure of Cry proteins suggest that domain I from Cry toxins have evolved from a common origin. The phylogenetic tree is composed of three branches. The first branch includes the domain I from lepidopteran specific toxins (Cry 1), the second branch comprised the domain I from the coleopteran specific toxins (Cry 3, Cry 7 and Cry 8) and also includes the dual specific, lepidopteran and coleopteran toxins (Cry 1B and Cry 11), the domain I from dipteran, nematode and sarcomastigophora specific proteins are included in the third branch. This topology showed a correlation of the type of domain I and the specificity of the toxin protein with which they are associated, suggesting that different types of domain I have been selected for acting in particular membrane conditions from the distinct target types.

In relation with these results, we are interested to study the differences in ion channel activities display by different Domains I. The analysis of different Cry I toxins revealed that in the lepidopteran group there are differences in permeability and selectivity among different Cry toxins.

We are planing to analyze the differences in mode of action (changes in permeability, selectivity and pH dependence) of different types of domains I and whole Cry toxins in dipteran (*Culex quinquefasciatus*) midgut-membranes.

P-116Th

IS AMINOPEPTIDASE N THE RECEPTOR OF Cry1Ac δ-ENDOTOXIN IN *Trichoplusia ni* MIDGUT ?

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Cry1Ac is one the members of the insecticidal crystal proteins family from *Bacillus thuringiensis*. After solubilization and proteolytic activation, Cry toxins have been shown to bind to specific high affinity binding proteins (receptors) on the surface of midgut epithelial cells. The generally accepted model for Cry toxins mechanism of action is that following binding, the toxin undergoes a conformational change and inserts into the membrane. An oligomer formed by several toxin molecules then forms cation-selective channels that leads to cell lysis and eventually to the insect death. The receptor for Cry1Ac in *Manduca sexta*, *Heliothis virescens*, *Lymantria dispar* and *Plutella xylostella* has been identified as a 120 kDa aminopeptidase N (APN). At least a proportion of APN is anchored in the membrane by a glycosylphosphatidylinositol (GPI) anchor. It has been shown *in vitro* that N-acetyl-D-galactosamine (GalNAc) blocked the binding between Cry1Ac and the APN.

In this study membrane potential measurements using a fluorescence dye (dis-C_3-(5)) suggest that APN also could be involved as receptor in the *T. ni* midgut because: 1) 250 μM GalNAc was able to inhibit all the Cry1Ac pore-forming activity. 2) There are no Cry1Ac effect on brush border membrane vesicles (BBMV) preincubated with a GPI-specific phospholipase C (PIPLC). 3) The K^{+}-toxin induced permeability increase depends on the quantity of APN: 22 % K^{+}-permeability increase on BBMV with 4 fold APN compared to 55 % K^{+}-permeability increase on BBMV with 12 fold APN. It was found that exhaustive PIPLC digestion of *T. ni* BBMV released approximately 75 % of total APN. The APN-PIPLC resistant apparently also interacts with Cry1Ac toxin, because when the toxin concentration was increased 4 fold (0.2 to 0.8 pmol/μg BBMV) a pore-forming activity was recovered. The kinetic constants (Km and Vmax) and the Vmax/pH profiles calculated by the APN-PIPLC sensitive and resistant indicate that they are different enzymes.

P-117Th

THE INSECTICIDAL CRYSTAL PROTEIN FAMILY FROM *Bacillus thuringiensis* .

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The insecticidal crystal proteins (ICPs or Cry) are a family of proteins that have biocidal activities against very different targets. The ICPs mode of action is a multistage process. First, the crystals ingested by a susceptible larva dissolve in the alkaline environment of the gut, releasing soluble proteins. The inactive protoxins are cleaved at specific sites by proteases yielding 60-70 kDa protease-resistant, active toxin fragments. The activated toxin binds to receptors located on the apical microvilli membrane of epithelial midgut cells. Specificity is mainly attributed to the interaction of ICPs with their receptors in the insect gut microvilli. After the toxin binds the receptor, a change in the toxin conformation is thought to occur, allowing toxin insertion into the membrane. Oligomerization of the toxin follows, and this oligomer then forms a pore that leads to osmotic cell lysis.

The ICPs are modular in structure, consisting of three different functional domains. Experimental data from several laboratories have shown that domains from Cry proteins are structural independent. Isolation of domain I and α-helix peptides retains its ability to form cation channel formation in planar lipid bilayers. We will present that isolated Domain II-III is able to interact with the membranes of lepidopteran insect in a similar way as the complete toxin.

Several proteins are organized as discrete modules which may have different functions. It has been proposed that domain swapping may contribute to the versatility of protein function and therefore be an important molecular mechanism for their evolution. In this work, the evolutionary relationships of the Cry protein family will be presented. The phylogenetic analysis of the Cry toxin family shows that the great variability in the biocidal activity of this family has resulted from two fundamental evolutionary process. First, the independent evolution of the three functional domains, and second, domain swapping among different toxins. These two processes have generated proteins with similar mode of action but with very different specificities.

The participation of each domain in the mode of action of the complete toxin will be reviewed in relation to the phylogenetic analysis.

P-118Th

CHEMICAL CONVERSIONS OF THE TUMOR PROMOTING MYCOTOXIN FUMONISIN B1 FROM FUSARIUM MONILIFORME DURING FOOD PROCESSING

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The fumonisins are a series of sphingosine-analog mycotoxins produced in large amounts by *Fusarium moniliforme*, a ubiquitous contaminant of stored maize (corn) worldwide. The toxins are of interest because they are environmental tumor promoters which are sufficiently widespread to potentially play an important role as a cause of human cancer. Fumonisins possess sufficient thermal stability that normal processing of maize-derived foods reduces, but does not completely eliminate FB_1 detectable by sensitive HPLC assays. Fumonisins are similar in structure to sphingosine except they lack a hydroxyl group on the carbon adjacent to the one bearing the amino group, and the fatty acid chain is modified by methylation and hydroxylation, as well as the presence of two esterified propanetricarboxylic acid side chains. However, structure-activity relationship studies have indicated that none of the fatty acid chain modifications are required for biological activity, including the sidechains, which constitute about half the molecular weight of the toxin. Thermal processing (roasting, frying) of maize containing added biosynthetically-radiolabelled FB_1 resulted in up to 50% of the toxin becoming covalently bound to a protein-containing fraction. The chemical nature of this linkage has not yet been established, but hydrolysis of the protein derivative releases the biologically active toxin backbone (hydrolyzed FB_1). The likelihood that a similar release of active toxin would occur during digestion of protein-toxin conjugates in the intestinal tract means that more research must be done before the threat to human safety posed by fumonisins can be assessed. The major reaction of FB_1 on heating in dry form appears to be loss of water from a side chain yielding an anhydride which may then react with amino or thiol groups in proteins giving covalently bound toxin. Some other plausible conversions of FB_1 on heating appear to be less favored reactions. Very low yields were obtained for ester pyrolysis to olefin derivatives during heating in a dry form. Similarly, heating FB_1 with xylan or xylose in an aqueous environment that should generate Maillard degradation products was not an effective method to produce 3-hydroxypyridine derivatives of FB_1. (Supported in part by USDA/NRICGP grants 93-37201-9561 and 96-35201-3271)

P-119Th

CHARACTERIZATION OF A LETHAL AND PARALYSING TOXIN (ACYL POLYAMINE) ISOLATED FROM THE VENOM OF *SCAPTOCOSA RAPTORIA* SPIDER.

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A great variety of toxins present in spider venoms are responsible for the arresting of their preys.They were characterized as hyaluronidase,insecticide peptides and acyl polyamines.

A biologically active acyl polyamine was isolated from the venom of *Scaptocosa raptoria* by three chromatographic steps: Sephadex G-10 gel filtration and two purification steps by reversed-phase HPLC.The acyl polyamine has a molecular weight of 2.200 Daltons, as determined by mass spectrometry.

The purified toxin caused flacid paralysis and had a rapid lethal effect on barbiers,cocroaches and crickets,as well as on spiders such as *Scaptocosa raptoria*, when injected into insect torax.The isolated acyl polyamine also presented bacteriostatic effect on *E.coli* O55 grown on minimum media and a fungicistatic effect on Candida albicon and Cryptococcus neoformans, but was not toxic to mice.

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P-120Th

CLINICAL FEATURES OF ENVENOMING BY *Bothrops leucurus* SNAKEBITE IN METROPOLITAN REGION OF SALVADOR, BAHIA, BRAZIL

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The *Bothrops leucurus* snake known as "jararaca-do-rabo-branco" has large distribution in the state of Bahia, being responsible for all the registered cases at the metropolitan region of Salvador. The present investigation is a descriptive study about the clinical aspects of the ophidian accidents caused by *B. leucurus* in the metropolitan area of Salvador, Bahia, Brazil, from January 1982 to April 1996. These accidents were notified to the Information Centre of Antivenom (CIAVE). This study is based on medical records of 126 patients. Envenomation by *B. leucurus* is mainly characterized by: local disturbance manifestations: pain (80.9%), edema (79.8%), heat (26.3%), numbness (20.2%), erythema (16.7%), ecchymosis (13.2%) and phlycthena (10.5%) and coagulation manifestations: period of altered coagulation and blood incoagulability (58.2%). General manifestations such as headache (14.0%) and dizziness (7.9%), vomiting (4.4%) and nausea (3.5%), oliguria (3.5%), haemorrhage (3.5%), hypotension (1.8%), reduced vision acuity (1.8%) and tremors (1.8%) were observed with less frequency. Most of the envenomation cases were mild (52.1%) and all evolved to cure. This might have direct relation to time between the snakebite and the medical care, therefore the gravity of the case increased with the time. The high frequency of mild cases may also be related to snake length, however the majority of the accidents were caused by young snakes. On average, 7.5 ampoules/patient were used, as the majority of the patients received 1 to 4 ampoules (50.5%). The were administered, on average, 5.0 ampoules in mild cases, 7.4 ampoules in moderate cases and 8.8 ampoules in severe cases. The absence of the lethality and the high frequency of mild cases with no sequels suggest that the treatment with bothropic antivenom effectively neutralized the action of venom, indicating the efficiency of this treatment of accidents in the metropolitan area of Salvador.

P-121Th

INCREASE OF VASCULAR PERMEABILITY IN RAT INDUCED BY *Micrurus lemniscatus* VENOM

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Micrurus venoms cause mainly neurotoxic effects both clinically and experimentally. In addition, several reports show that coral snake venoms induce local effects such as myotoxicity and edema in experimental animals. In the present study we evaluated the effect of two populations of *Micrurus lemniscatus* snake crude venoms (ML-I and ML-II) on the vascular permeability of rat. This effect was investigated 5, 15, 30 and 60 minutes after i.v. injection of ML venoms (0,5 mg/kg) or Ringer-Locke solution into male Wistar rats. Histamine (5 µg) or serotonin (1 and 5µg) i.d. injection were used as positive controls. Vascular permeability to plasma proteins was assessed by measuring extravasation of Evans blue dye (EB, 20 $mg.kg^{-1}$, i.v.) into the skin of animals. The dye was extracted from tissues with formamide and its concentration determined by spectrophotometry (620 nm). *ML-I* and *ML-II* i.d. injections caused increase of vascular permeability at all doses studied, ML-I being more potent than ML-II and histamine. The peack of increase of vascular permeability was detected 15 minutes after the venom injection. In order to understand the mechanism of ML-I-induced effect on the rat skin, groups of animals were treated with promethazine (5 mg/kg, i.p.) and methysergide (5 mg/kg) or saline (i.p.) 30 minutes before injections of ML-I. The ML-I-induced increase of vascular permeability was significantly reduced by promethazine and methysergide treatments, with 63 and 41% reduction, respectively. These results suggest that the increase of vascular permeability induced by *Micrurus lemniscatus* venom is, at least, partially mediated by activator of histamine (H_1 type) and serotonin vascular receptors. This activation of receptors may be triggered by ML-I-induced release of mediators from mastocites and/or by some components with autacoid-like effect present in the *Micrurus lemniscatus* venom. **Financial Support**: CNPq

P-122Th

RECRUITMENT OF NEUTROPHILS AND RELEASE OF INFLAMMATORY MEDIATORS INDUCED BY *Tityus serrulatus* VENOM (TsV) IN THE LUNG OF RATS.

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Several experimental and clinical data showed that *Tityus serrulatus* envenomation causes severe pulmonary edema and injury. The mechanisms leading to these effects are not completely understood. In the present study the lung injury induced by TsV was characterized in terms of leukocytes influx and release of inflammatory mediators. The infiltration of leukocytes was evaluated by counting cells in the bronchoalveolar lavage fluid (BALF) and by measuring neutrophil myeloperoxidase activity in homogenates of lung tissue. Concentrations of LTB_4, TXA_2, PGE_2 and TNF-α in the BALF were assessed by specific ELISA and citotoxicity on L929 cell line, respectively. Injection of TsV (200 µg/kg, i.v.) into adult male Wistar rats caused a significant leukocytes influx, mainly neutrophils, in the BALF from 4 to 48 h. Neutrophils were also found in the lung parenchyma between 2 and 6 h. Increased levels of TXA_2 and PGE_2 were detected in the BALF from 15 to 30 min after TsV injection whereas those of LTB_4 increased from 1 to 4 h. TNF-α levels were not affected. The present results show that i.v. injection of TsV induces inflammation in the lung of rats. This reaction is characterized by a marked infiltration of neutrophils into lung parenchyma followed by their accumulation into the bronchoalveolar space and release of lipid mediators. Release of potent chemotactic substances, such as LTB_4 and TXA_2 may account for the TsV-induced recruitment of leukocytes. Moreover, since PGE_2 potentiates the increase of vascular permeability caused by several mediators a contribution of this eicosanoid for the lung edema is suggested. In view of the ability of neutrophils to release toxic oxigen-derived metabolites and granule associated enzymes under uncontrolled stimulation it is reasonable to suggest that these cells play a role in the genesis of the TsV-induced lung injury.**Financial support:** FAPESP; Fundação Instituto Butantan.

P-123Th

FUNCTIONAL ALTERATIONS INDUCED IN PERITONEAL MACROPHAGES BY MYOTOXINS ISOLATED FROM *Bothrops asper venom* (BaV).

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Several myotoxins phospholipases A2 (PLA2) have been purified from BaV. Among them, myotoxin II (MII) is a Lys 49 PLA2 homologue devoid of catalytic activity, and myotoxin III (MIII) is an Asp 49, enzymatically active variant. Besides their ability to induce myonecrosis, these PLA2s are cytotoxic to a variety of cell types *in vitro* and induce edema. To further investigate the pharmacologycal actions of these myotoxins, their effects on peritoneal macrophages were studied. Peritoneal macrophages were obtained from Swiss mice previously injected i.p. with 4% tioglycollate. Macrophages were incubated with either MII, MIII or PBS (controls) at various toxin concentrations (1.5, 3, 6, 12, 25 and 50 µg/ml) and the following parameters were investigated: Cytotoxicity (by Trypan blue exclusion), H_2O_2 production (by the phenol red method) and spreading activity on coverslips. Myotoxins induced cytotoxicity only at the highest dose tested (50 µg/ml), MIII begin more cytotoxic than MII. On the other hand, both myotoxins induced an increment in H_2O_2 production when macrophages were stimulated concomitantly with PMA (10ng/ 2 x 10^5 cells), although no increment in H_2O_2 production upon incubation of macrophages and myotoxins without the addition of PMA. With PMA stimulation, MIII enhanced H_2O_2 production at all doses tested, whereas MII increased H_2O_2 production only at the dose of 6 µg/ml. Macrophages spreading activity was inhibited by myotoxins, being the effect more pronounced after incubation with MIII. It is conclued that MII and MIII affect macrophages functions at subcytotoxic doses. Such effects are not strictly dependent on PLA2 activity, although enzymatic action seems to enhance them. Effects observed on H_2O_2 production and cellular spreading suggest that different mechanisms are involved.
Financial support: FAPESP

P-124Th

HYPERALGESIA INDUCED BY *Bothrops asper* VENOM (BaV) AND MYOTOXIN III IN RATS

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BaV induces prominent local tissue damage (myonecrosis, hemorrhage, edema and pain). Despite its clinical relevance, venom-induced pain and hyperalgesia have been poorly investigated. This study assessed the hyperalgesia induced by BaV and myotoxin III (MIII), an Asp-49 myotoxin phospholipase A2 isolated from this venom. Male Wistar rats were injected in the subplantar region of the right hindpaw with BaV, MIII os saline (control). Pain threshold was assessed before injection and 1,2,4,6 and 24hr after injection with an analgesimeter UGO BASILE. Pain threshold was expressed as the weight (g) which caused the withdrawal of the paw. Both BaV and MIII, at doses of 5, 10 and 15µg/rat, increased the sensitivity to pain after the first hr. Hyperalgesia induced by BaV peaked at 2hr and decreased afterwards, reaching values similar to those of control rats at 24hr. MIII induced hyperalgesia of a similar time-course, although the peak was observed at 1 hr. Maximum responses were observed with 15 µg of BaV and 10µg of MIII (45% and 40% of reduction in pain threshold, respectively). Pretreatment with dexamethasone (0,4mg/kg, p.o., 90min before venom or toxin injection) reduced BaV-induced hyperalgesia by 64% and abrogated hyperalgesia induced by MIII. NDGA (30mg/kg, i.p., 30min before injection) blocked hyperalgesia induced by both venom and toxin. On the other hand, indomethacin (2mg/kg, i.v.) and BN 52021 (5mg/kg, i.v.) did not affect hyperalgesia when administered 30 min before envenomation. BaV and MIII induce hyperalgesia in rats. This effect seems to depend on phospholipase A2 activity and is probably mediated by leucotrienes.
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P-125Th

EFFECT OF *BOTHROPS ASPER* VENOM (BaV) ON LEUKOCYTE-ENDOTHELIAL INTERACTIONS. A DIRECT VITAL MICROSCOPIC STUDY.

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BaV affects blood coagulation and evokes prominent local tissue damage with myonecrosis, hemorrhage and inflammatory response. It has been demonstrated that this inflammatory reaction is characterized by multifactorial edema and leukocyte infiltrate. This work was performed to study the mechanisms involved in leukocyte recruitment in this envenomation. The leukocyte-endothelial interactions, initial events that allow the extravasation of leukocytes into inflamed areas, were determined in postcapillary venules of the internal spermatic fascia of Wistar rats. The diameter of the venules and the number of rolling and sticking leukocytes were determined after topic applications of different doses of BaV. Results showed that: 1) the application of 1 µg of BaV did not cause significant alterations on any of the parameters studied; 2) the administration of 5 or 10 µg of the venom induced a significant increase in the venule diameter associated with blood coagulation disturbances, evidenced by thrombi, that evolved to stasis; 3) using an intermediate dose, 2 µg, a significant vasodilatation and a marked increase in the number of rolling and sticking leukocytes was observed. Fibrin clots were also noted, although without stasis. These data suggest a possible interrelation between venom effects determining leukocyte recruitment. The leukocyte-endothelial interactions seem to be influenced by other effects besides inflammatory mediation, such as alterations in blood coagulation.

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P-126Th

CHARACTERIZATION OF LOCAL TISSUE DAMAGE EVOKED BY *BOTHROPS JARARACA* VENOM (BjV) IN THE RAT CONNECTIVE MICROCIRCULATION. AN INTRAVITAL MICROSCOPIC STUDY.

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BjV induces blood coagulation disturbances besides a pronounced local lesion characterized, mainly, by hemorrhage and inflammatory reaction. Despite these effects being well described, the mechanisms involved and the kinetic of the events are not completely known. Then, the aim of this study was to investigate the effects of topic administration of BjV on the microcirculation of internal spermatic fascia of Wistar rats. After application of 1 or 10 µg of the venom, the following events were investigated: 1) alterations of postcapillary venules diameters and 2) the formation of hemorrhagic lesions. Results showed that application of 10 µg of BjV induced a rapid increase on vessel diameter and a marked alteration on local blood coagulation that developed to stasis in few minutes. Hemorrhage appeared after 3 min of administration originating, preferentially, from capillaries and postcapillary venules. The administration of 1 µg of BjV also induced a vasodilatation however did not evoke significant blood-flow disturbances. Hemorrhage was visualized in capillaries only after 20 min of application of BjV. The results suggest that different doses of the venom evoke distinct intensity of response, possibly involving different mechanisms of action.

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P-127Th

MYOTOXIC ACTIVITY OF AN ACIDIC PLA_2 FROM *Lachesis muta* SNAKE VENOM

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Phospholipases A_2 are enzymes present in some snake venoms which can be classified by their primary structure and cell location. They also present several pharmacological properties, such as hemorraghic, myotoxic, and neurotoxic effects. Recently, we have purified an acidic PLA_2 from *Lachesis muta* snake venom (LM-PLA_2) with apparent molecular weight of 17 kDa and displaying inhibitory effects upon platelet aggregation induced by several physiological agonists. The N-terminal sequence of LM-PLA_2 presented an aspartic acid residue at position 49 and a high homology with others PLA_2s. The high enzymatic activity of this enzyme corroborates this property as characteristics for the D-49 group of PLA_2s. No hemorraghic, toxic or anticoagulant effects were detected for this protein. However LM-PLA_2 incubated in vitro with mice muscle , induced a dose and time-dependent increase on rate of release of creatine kinase (CK) and in vivo, increase the basal levels in plasma CK activity in treated animals. Furthermore, we observed that treatment LM-PLA_2 with p-bromophenacil bromide abolished in parallel its enzymatic and myotoxic activities, suggesting that the latter one depends on the catalytic properties of LM-PLA_2. On the other hand, LM-PLA_2 myotoxic effect was not abolished by heparin, in contrast to what has been described for several others basic PLA_2s presenting myotoxic activity.

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P-128Th

THE STUDY OF ANALGESIC EFFECT OF NEUROTOXINS FROM *NAJA NAJA ATRA* VENOM

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By using the methods of mice hot-plate method, K+-electronic stimulate permeation method and acetate twist-body pain detecting method, the analgesic effects of neurotoxin were investigated. The results showed that neurotoxin had apparent analgesic effect ($P<0.05$-0.01). 1. Mice hot-plate method showed that neurotoxin had obvious analgesic effect after administration (0.06-0.24 mg/kg orally or 0.03 mg/kg subcutaneous injection) for 2hr. Compared with morphine (6.0 mg/kg), the neurotoxin took effect slowly (2hr after), but last a longer time (>12 hr). 2. Experiment of rat K+-electronic stimulate permeation method showed that both neurotoxin (0.07-0.3 mg/kg orally or 0.03 mg/kg subcutaneous injection) and morphine (6.0 mg/kg) had equal analgesic effect. Morphine took effect quickly 1hr after and disappeared in 6-8 hr. But neurotoxin took effect slowly and lasted 12-24 hr. 3. Experiment of acetate twit-body pain detecting method showed that the number of body twist was reduced 30 min after administration of neurotoxin (0.05-0.1 mg/kg orally), and had apparent effect-quantity relationship. The effect was equal to aspirin (100 mg/kg) and better than dulantin (30 mg/kg). 4. After injection of neurotoxin 0.08 mg/kg /day for 4 days and 0.01 mg/kg/day for 3 days, the concentration of enkephalin in rat brain were tested. Enkephalin was obviously high than control group ($P<0.01$). 5. Injection neurotoxin 0.03 mg/kg for10 days, the analgesic effect increased against saline group ($P<0.01$).

P-129Th

CHARACTERIZATION OF A PROTHROMBIN ACTIVATOR FROM *AGKISTRODON HALYS PALLAS* VENOM

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The procoagulant and anticoagulant actions of *Agkistrodon halys pallas* venom were examined with human plasma in the presence and in the absence of calcium ions. The venom was tested in its native form, after treatment with a specific and irreversible inhibitor of serine proteases (phenylmethanesufonyl fluoride), or after treatment with a specific and irreversible inhibitor of PLA_2s (*p*-bromophenacyl bromide). The effect of the venom on hemostasis was further characterized by measuring its ability to activate purified blood coagulation factors. A prothrombin activator (aharin) was purified from the venom. It consists of a single polypeptide chain, with a mol. wt of 63,000. Aharin possessed no amidolytic activity on chromogenic substrates. It did not act on other blood coagulation factors, such as factor X and plasminogen, nor did it cleave or clot purified fibrinogen. Our results indicate that different components of *A. halys pallas* venom activate blood coagulation at the two final steps of the coagulation cascade. Thus, in addition to the fibrinogen clotting enzyme, which converts fibrinogen into fibrin directly, we characterized a prothrombin activator in the venom. This prothrombin activator is very similar with those prothrombin activators from *Echis carinatus* and *Bothrops atrox* venoms in biochemical and enzymatic properties.

P-130Th

MECHANISM OF THE EFFECT OF A PHOSPHOLIPASE A_2 PLATELET INHIBITOR FROM *OPHIOPHAGUS HANNAH* VENOM

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An acidic phospholipase A_2 (OHV A-PLA_2) from *Ophiophagus hannah* venom has been shown to be a potent inhibitor of platelet aggregation. The antiplatelet effects of OHV A-PLA_2 did not increase when preincubation times of platelets and OHV A-PLA_2 were prolonged indicating phospholipid hydrolysis did not significantly contribute to the antiplatelet effects. Alkylation of active site His residue using *p*-bromophenacyl bromide resulted in complete loss of enzymatic activity, but the modified enzyme retained more than 30% of its antiplatelet effects. Platelet free intracellular calcium was measured by flow cytometry, and the result revealed that (OHV A-PLA_2) induced an apparent increase in intracellular calcium in resting platelets. Ultrastructural studies showed that OHV A-PLA_2 induced remarkable morphological changes both in the shape and the subcellular structures. There were disorders in the open canalicular system and dense tubular system, indicating a dramatic change in the "cell sarcoplasmic reticulum". ATP-release of platelet induced by various inducers was also inhibited after platelets treated with OHV A-PLA_2. We conclude that OHV A-PLA_2 inhibited platelet aggregation by the mechanism independent of the enzymatic activity. The intracellular calcium overload, and the disorder of the cytoskeleton and sarcoplasmic reticulum leading to the loss of release reaction may play an important role in OHV A-PLA_2 inhibiting platelet aggregation.

P-131Th

THE ACTION OF *Bothrops neuwiedi* VENOM ON CHICK *BIVENTER CERVICIS* AND MOUSE PHRENIC NERVE-DIAPHRAGM PREPARATIONS.

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We have examined the neuromuscular activity of *Bothrops neuwiedi* venoms in nerve-muscle preparations. Directly and indirectly stimulated chick *biventer cervicis* and mouse phrenic nerve-diaphragm preparations suspended in Tyrode solution were exposed to varying concentrations of crude venom and its fractions (obtained by chromatography on Sephacryl S-200 HR) for up to 120 min. In the *biventer cervicis* preparation, *B. neuwiedi* venom (50 μg/ml; n=4) initially increased the twitch-tension amplitude in response to indirect stimulation before blocking neuromuscular transmission. At 5 μg/ml, the venom irreversibly blocked the twitch-tension response (93%; n=4) as well as KCl (13.4 mM)-induced contractures (80%; n=4). At this venom concentration, there was no effect on the contractile response to ACh (14.6 mM; n=4), although at higher doses (>50 μg/ml) inhibition was observed (n=3). In curarized *biventer cervicis* preparations (d-TC, 8 μg/ml), *B. neuwiedi* venom irreversibly blocked the muscle response to indirect (90% blockade at 10 μg/ml; n=4) and direct (60% inhibition at 50 μg/ml; n=3) stimulation. The neuromuscular activity was recovered in fractions 53 to 57 (peak 1) and 81 to 84 (peak 2) of the fractionated venom. All of the tested fractions totally blocked the KCl responses (n=17), with relatively little effect on those to ACh (20% inhibition; n=17). The most active fraction induced 50% paralysis in 45 min. In the mouse phrenic nerve-diaphragm preparation, the responses to indirect and direct stimulation were inhibited only at >20 μg/ml (70%; n=7) and ≥50 μg/ml (80%; n=5), respectively. These results suggest that, at least at low doses (5-20 μg/ml), *B. neuwiedi* venom acts principally at presynaptic sites.

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P-132Th

Envenomation of children by snakes of the genera *Bothrops* and *Crotalus*.

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A retrospective study was performed in order to evaluate the clinical outcome of 97 children under 14 years old (median=9 years old) bitten by snakes of the genera *Bothrops* (70.1%) and *Crotalus* (29.9%), from January 1984 to April 1997. The severity of envenomations was classified according to the recommendations of the Ministry Health of Brasil. On admission, of the 68 children bitten by *Bothrops spp*, 29.4% were classified as mild envenoming, 50.0% as moderate envenoming and 20.6% as severe envenoming. Of the 29 children bitten by *Crotalus spp*, 13.8% were classified as mild envenoming, 31.0% as moderate envenoming and 55.2% as severe envenoming. Before the administration of antivenom, blood coagulation disorders were observed in 66.0% (incoagulable blood in 46.4%) of the 56 children bitten by *Bothrops spp* and in 82.6% (incoagulable blood in 78.3%) of the 23 children bitten by *Crotalus spp*. The main clinical complications observed after *Bothrops spp* bites were local infections (17.6%) (including cellulitis, 11.7%, and abscesses, 5.9%), compartmental syndrome (4.4%), gangrene (1.5%) and acute renal failure (1.5%). Most children bitten by *Crotalus spp* presented neurotoxic signs (86.2%), three children (10.3%) developed acute renal failure and one (3.4%), a severe systemic bleeding. No deaths were observed. In a geographical region where the main species and subspecies of these venomous snakes have been identified as being *B. jararaca* and *C. durissus terrificus*, we conclude that: 1- envenomation of children under 14 years old by *Crotalus spp* was significantly more severe than that caused by *Bothrops spp* (χ^2, $p<0.01$) and, 2- the frequency of blood coagulation disorders was significantly greater after bites by *Crotalus spp* than by *Bothrops spp* (χ^2, $p<0.05$).

P-133Th

LOCAL EFFECTS OF *Bothrops lanceolatus* VENOM

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We report here the edema and hemorrhage induced in rats by heated and non-heated *Bothrops lanceolatus* venom (BLV). Edema was induced by the subplantar injection of BLV (12.5 - 100 µg/paw) and quantified by hydroplethysmography. Hemorrhage was induced by injecting BLV (0.25 - 64 µg) into the gastrocnemius muscle and the extent of damage determined by the cyanomethemoglobin method. Edema and hemorrhage were maximal within 15 min and 1 h, and disappeared within 24 h and 72 h, respectively. Heating BLV reduced the edema by 70% and abolished the hemorrhage activity. The co-administration of iloprost (0.1 µg) or captopril (300 µg) with heated BLV potentiated the edema 200% and 44%, respectively. Pre-treating the rats with mepyramine, cyproheptadine, mepyramine + cyproheptadine, dexamethasone, BW A4C or Hoe 140, inhibited the edema induced by heated BLV coinjected with iloprost (0,1 µg) by 28%, 55%, 77%, 63%, 35% and 30%, respectively. Indomethacin had no effect on the edematogenic response. Pre-incubating non-heated BLV with anti-BLV antiserum completely neutralized the hemorrhagic activity but only abolished 50% of the edema. The i.v. administration of anti-BLV antiserum immediately after the subplantar or i.m. injection of venom neutralized the edema (30%) and the hemorrhage (38%). These results indicate that non-heated BLV induces paw edema and hemorrhage in rats while heated BLV induces edema without hemorrhage. The edema is attributable primarily to the formation or release of bradykinin, histamine, serotonin and lipoxygenase products. Anti-BLV antivenom neutralized the hemorrhagic and edematogenic activities better when it was pre-incubated with BLV than when it was administered independently i.v.

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P-134Th

CHARACTERIZATION OF THE NOCICEPTIVE AND EDEMATOGENIC ACTIVITIES OF THE *Thalassophryne nattereri* FISH VENOM

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Accidents caused by the poisonous fish *T. nattereri* are frequent on Brazilian shores, particularly in Northeast. The main signs of envenomation includes edema, erythema, necrosis of the affected member, fever and excruciating pain. This work investigated the nociceptive and edematogenic activities induced by subcutaneous injection of fish venom (FV) into the dorsal surface of the hind paw in mice. The nociceptive response was determined as the time spent by the animals licking or biting the injected paw. The edematogenic response was determined after 2 hr injection of FV, and measured as the difference (mg) between the right paw injected with FV and the contralateral paw injected with equal volume of saline. Subcutaneous injection of FV (30 µl; 3-200 µg/ml in saline) into the right hind paw induced nociception and edema in a dose related manner, reaching a maximum after injection of 100 µg (nociception: 223.3 ± 28.8 s and edema: 135.0 ± 6.8 mg, n=6). Previous treatment with the antiinflammatory agents. Indomethacin (30 min., 10mg/kg, i.p.) or Dexamethasone (60 min, 1 mg/kg, s.c.) did not affect either the nociceptive or edematogenic responses induced by 30 µg FV. Pretreatment with Fentanyl (30 min., 0.1 mg/kg, s.c.), an opioid analgesic, or phenylacetyl-FSR-EDDnp (TKI; 30 min., 100 mg/kg, i.p.), a tissue kallicrein specific inhibitor (Juliano *et al., Peptides* 1994: 885, 1995) reduced the nociceptive response by 93% and 78% of control, respectively. TKI also reduced the paw edema by 26% of control. The results indicate that the nociceptive and the edematogenic responses induced by *T. nattereri* fish venom were not affected by classical antiinflammatory agents, but they were reduced by the tissue kallikrein inhibitor, phenylacetyl-FSR-EDDnp. These data indicate that activation of the tissue kallikrein-dependent pathway is involved in the venom local effects.

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P-135Th

INFLAMATORY RESPONSE INDUCED BY *Micrurus frontalis altirostris* VENOM

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The main symptoms observed in the envenomation caused by the snake *Micrurus frontalis altirostris* are related to neurotoxic effects of the venom. However, the local reaction at the bite site has not been described yet. The inflammatory response induced by the venom of *Micrurus frontalis altirostris* was studied in mice. The following parameters were evaluated: the increase of vascular permeability (Evan's blue dye method), paw edema (pletismography) and cell migration after intraperitoneal injection. The intraplantar injection of 1.0 µg of the venom induced increase in vascular permeability which started a few minutes after the injection. This venom showed also a dose dependent edematogenic response. It was maximal 30 min after the injection, decreased gradually and completely disappeared by 24 hours. The venom did not induce a significant cell migration after the intraperitoneal injection of 3.0 µg in mice (4,8 and 24 hours).The results suggest that the venom of *Micrurus frontalis altirostris* alters some vascular events of inflammatory response, but not the cellular ones.

P-136Th

EVIDENCE THAT ANTINOCICEPTION INDUCED BY *Crotalus durissus terrificus* VENOM ON INFLAMMATORY HYPERALGESIA IS PARTIALY MEDIATED BY PERIPHERAL OPIOID RECEPTORS

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It has been demonstrated that the *Crotalus durissus terrificus* (Cdt) venom is endowed with antinociceptive effect when evaluated by the hot plate test and the acetic acid-induced writhing in the mouse. As this effect has been shown to be centrally mediated by opioid receptors, the aim of this work was to investigate the possible participation of peripheral opioid receptors in a model of inflammatory hyperalgesia. The rat paw pressure test, employing carrageenin (CA) as nociceptive stimulus, was used to measure inflammatory hyperalgesia. A force (in g) with increasing magnitude was applied to the paw and the values which induced the withdrawing of the paw represented the pain threshold. The test was applied before and 3 hs after the injection of CA. Cdt venom (200 µg/kg) or saline (control), were administered p.o. immediately before, 1 or 2 hs after the intraplantar (ipl) injection of CA (200 µg/paw). Cdt venom not only blocked the hyperalgesia induced by CA but determined an increase in pain threshold observed before the injection of CA. Naloxone was administered s.c. (1 mg/kg, 165 min after CA) or ipl (1 µg/paw, simultaneously or 165 min after CA). Results showed that the antinociceptive effect of the venom was antagonized by naloxone. When carrageenin was injected into both hind-paws and naloxone into one hind-paw, the effect of the venom was abolished in the paw injected with naloxone. These data suggest that peripheral and local opioid receptors are involved with the antinociceptive effect of the venom on inflammatory hyperalgesia.

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P-137Th

CROTALUS DURISSUS SNAKEBITE REVISITED.

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From January 1991 to December 1996 110 patients bitten by Crotalus durissus were admitted to Hospital João XXIII in Belo Horizonte, Minas Gerais. Patients were classified into 2 groups according to the interval between the bite and hospital admission (ΔT): group 1 (n= 75, ΔT ≤ 6 hr) and group 2 (n=35, ΔT > 6hr). Clinical and laboratory data from these patients were recorded on a proforma. Blood was sampled for biochemical and haematological analysis and for whole venom and crotoxin assays (ELISA) before antivenom treatment and at 1hr (T1), 6hr (T6), 12hr, (T12) and 24hr (T24) after the start of antivenom therapy The patients were treated with 100-200 ml of *Crotalus durissus* antivenom. Gender, age, dose of antivenom and the frequency of paresthesia at the site of bite, myalgia and palpebral ptosis did not differ between the two groups. Plasma whole venom and crotoxin concentrations were higher in group 1 patients and both were no longer detected after antivenom therapy. Group 2 patients presented higher values of plasma creatine kinase enzyme activity and partial thromboplastin time and a higher incidence of acute renal failure. The incidence of acute respiratory failure did not differ between the two groups. Mortality rate was 4.5% and deaths occurred only in group 2 patients. Data from this series show that group 2 patients in spite of the lower plasma whole venom and crotoxin concentrations were more severely envenomed than group one patients suggesting that most of the injected venom had enough time to bind to cell receptors and to damage the tissues. Every effort should be carried out to treat patients bitten by *Crotalus durissus* within the first 6 hours after the snake bite.

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P-138Th

EFFECTS OF THE METALLOPROTEINASES, MUTALYSIN I AND II ON THE HUMAN BLOOD COAGULATION AND FIBRINOLYSIS SYSTEMS.

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The zinc endopeptidases, mutalysin I and II have been previously isolated from the bushmaster snake venom (*Lachesis muta muta*). The purified proteins represent 12 and 1% respectively of the hemorrhagic activity in the crude venom. Mutalysin I (100 kDa, two subunits) and mutalysin II (22.5 kDa, single chain) hydrolyse the Aα>Bβ chain of fibrinogen without clot formation. The specific fibrinogenolytic activity was estimated as 25.5 and 13.0 mg of fibrinogen/min/mg protein for mutalysin II and I respectively. *In vitro*, the mutalysins act directly on fibrin and they are not inhibited by serine proteinase inhibitors (SERPINS). Analysis by SDS-PAGE of fibrin hydrolysis by both metalloenzymes showed that mutalysin II (0.22 μM) digested completely the α, β and γ chains. On the other hand, mutalysin I (0.56 μM) hydrolysed selectively the α chain of fibrin leaving the β and γ chains unaffected. In contrast with the plasminogen activator - based thrombolytic agents (e.g.,streptokinase), mutalysin I and II do not activate plasminogen. Mutalysin II has no effect on protein C activation and does not inhibit platelet aggregation in human PRP stimulated by ADP or collagen. Incubation of mutalysin II with the primary plasma proteinase inhibitor α2-macroglobulin (α2-M) completely inhibited the dimethylcasein hydrolysis. The stoichiometry of inhibition was 1.0 mol of enzyme per mol of α2-M. Thus, in addition to hemorrhagic effect by direct proteolysis on protein components of capillary walls, the hemorrhagic toxins can digest other proteins of the haemostatic system and also activate endogenous proteinases.

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P-139Th

SNAKE BITE MORTALITY IN COSTA RICA

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The mortality rate due to snake bite envenomation in Costa Rica was estimated from 1952 to 1993. The highest mortality was observed during the fifties and sixties, with the highest rate of 4.83 per 100,000 population in 1953. In contrast, a rate of 0.2 per 100,000 population per year was estimated from 1990 to 1993. The most conspicuos decline in mortality ocurred after 1970. The highest mortality rates were observed in the provinces of Limón and Puntarenas, especially in regions where tropical rain forests had been transformed into agricultural fields. The lowest mortality was in the province of Guanacaste, where tropical dry forest predominates and *Bothrops asper* (terciopelo), the most important poisonous snake in the country, is not abundant. The majority of fatalities ocurred in the age groups from 10 to 19 years old. Males were more affected than females in a ratio of 3.6 to 1. Before 1980 most fatal cases did not received medical attention in hospitals, whereas after 1980 the majority of cases with fatal outcome were attended in hospitals.

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P-140Th

ENVENOMATION OF MICE BY THAI COBRA (NAJA KAOUTHIA) VENOM: TOLERABLE VENOM CONCENTRATION AND EXPOSURE TIME

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Tolerable venom concentration in circulation and tolerable exposure time are important indeces for development of a simple and rapid diagnostic method determining the causal venomous snake. We have recently examined venom concentration and time bringing mice to fatal conditions using venom of Thai cobra (N. kaouthia) , one of the most dangerous snakes in Thailand.

N. kaouthia venom appeared in circulation rapidly after intramuscular injection. The venom concentration attained a maximum level with all doses examined after 20 min. The half value of the maximum level was attained 1 min after injection when a dose of $4LD_{50}$ was used. A critical venom concentration endangering mice was assessed from venom concentration in the sera of mice envenomed with sublethal dose (LD_{50}). A fatal condition was produced within 30 min at a venom concentration of 200 - 300ng/ml or within 50 min at a venom concentration of 100 - 150ng/ml. A logarithmically proportional relationship was obtained between venom concentration and time to death.

P-141Th

Comparative study of the cytolytic activity of myotoxic phospholipases A_2 on murine endothelial and skeletal muscle cells *in vitro*.*

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A rapid *in vitro* cytolytic effect of myotoxic phospholipases A_2 (PLA_2s) isolated from snake venoms has been described. This study investigated if cytolytic activity is a common property of myotoxic proteins of this group. Murine endothelial cells (tEnd) and skeletal muscle (C2C12) myoblasts/myotubes were utilized as targets. As a measure of cell damage, the release of lactic dehydrogenase was determined 3 hr after exposure of cells to the PLA_2s, including representatives from the genera *Bothrops*, *Agkistrodon*, *Trimeresurus*, *Crotalus* (Viperidae), and *Notechis* (Elapidae). All of the group II myotoxic PLA_2s tested displayed rapid cytolytic activity, in the micromolar range of concentrations (8-32 μM). Myotubes, obtained after fusion and differentiation of C2C12 myoblasts, were significantly more susceptible to cytolysis than endothelial cells, previously reported to be more susceptible than undifferentiated myoblasts. Asp-49 and Lys-49 PLA_2 variants showed comparable cytolytic effect, suggesting that an intrinsic enzymatic activity of the toxin is not crucial to the mechanism of cell damage. Cytolytic activity appears to be a common characteristic of group II myotoxic PLA_2s of the Viperidae, while it is absent in notexin (group I myotoxic PLA_2) from Elapidae, and in the "small" (5 kDa) myotoxins, crotamine and myotoxin *a*. Such differences in cytolytic effect among myotoxins suggest differences in their mechanisms of action.

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P-142Th

Lysine-49 phospholipase A_2 and its synthetic peptide 115-129 kill bacteria

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Group II phospholipases A_2 (PLA_2s) of inflammatory fluids display bactericidal properties dependent on their enzymatic activity. A lysine-49 PLA_2 (myotoxin II) from the venom of *Bothrops asper*, which lacks the characteristic enzymatic activity of PLA_2s, retains direct lethal properties over a broad spectrum of bacteria. Its synthetic cationic peptide 115-129, which resides near the C-terminal loop, was able to reproduce the lethal effects of the intact protein. The direct bactericidal activity of myotoxin II, and that of its enzymatically active PLA_2 isoform (myotoxin III), were reduced by antibodies raised against the synthetic peptide 115-129. Heparin inhibited the bactericidal action of peptide 115-129. Following exposure to the peptide, accelerated uptake of the hydrophobic probe N-phenyl-N-naphtylamine was observed in sensitive but not in resistant bacteria, indicating that the lethal effect was initiated on the bacterial membrane surface. The outer membrane, isolated lipopolysaccharide (LPS), and lipid A of sensitive bacteria showed higher affinity for peptide 115-129 than the corresponding moieties of resistant strains. Lethality assays on bacterial-LPS chimeras indicate that LPS is a primary target for peptide 115-129: when heterologous LPS of the resistant strain was present in the context of sensitive bacteria, the chimera was resistant, and vice versa. The high affinity of peptide 115-129 for the outer membrane and isolated lipoteichoic acid of *Staphylococcus aureus* was commensurate with its sensitivity to the peptide. Myotoxin II and its peptide induced a morphologically similar bacterial cell damage in sensitive bacterial-LPS chimeras, which paralleled that observed in sensitive strains. A bactericidal mechanism independent of enzymatic activity is proposed.

P-143Th

LOCAL TISSUE DAMAGE INDUCED BY BAP1, A METALLOPROTEINASE ISOLATED FROM *Bothrops asper* SNAKE VENOM

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The pathological effects induced by the metalloproteinase BaP1 were studied as an approach to understand the mechanism of hemorrhage and the role of this toxin in the induction of local tissue damage. Vital microscopy using mouse *cremaster* muscle evidenced the formation of multiple hemorrhagic foci, originating from capillaries and small venules. Histological observations in mouse *gastrocnemius* muscle showed prominent hemorrhage few min after injection and areas of myonecrosis several hours after BaP1 injection. Myonecrosis was followed by an incomplete regenerative response. BaP1 was not cytotoxic to endothelial cells in culture, causing only a mild detachment from the culture plate. BaP1 hydrolyzed types I and IV collagen, fibronectin and laminin upon incubation with these extracellular matrix proteins *in vitro*. When injected i.m., BaP1 also induced skin lesions characterized by blistering. Histological observations indicated that blisters result from the separation of the epidermis from the dermal-epidermal junction. Elevated levels of matrix metalloproteinases were detected by gelatin zymography in skin homogenates and inflammatory exudate. BaP1 was detected in exudate 1 and 6 hr after i.m. injection. These results suggest that BaP1 is not directly cytotoxic to endothelial cells *in vitro* and that hemorrhage may be due to proteolytic degradation of basement membrane components of microvessels. Moreover, the toxin contributes to local tissue damage by inducing myonecrosis, blistering, inflammation and extracellular matrix alterations. It is suggested that release and activation of endogenous proteases may play a role in local tissue damage induced by BaP1.

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P-144Th

PHARMACOLOGICAL MODULATION OF EDEMA INDUCED BY LYS-49 AND ASP-49 MYOTOXIC PHOSPHOLIPASES A_2 ISOLATED FROM THE VENOM OF THE SNAKE *Bothrops asper* (TERCIOPELO)

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The pharmacological modulation of edema-forming activity of *Bothrops asper* myotoxins II and III, Lys-49 and Asp-49 phospholipases A_2, respectively, was studied plethysmographically in the mouse foot pad model. Animals were pretreated with diphenhydramine, dexamethaxone, indomethacin and prazosin 30 min before toxin injection, and edema was evaluated at 30, 60 and 140 min. Myotoxin III had phospholipase A_2 activity, whereas myotoxin II was devoid of enzymatic activity. Both toxins induced a dose-dependent edema of rapid onset, myotoxin III having a higher activity. Pretreatment of animals with diphenhydramine, dexamethasone and prazosin significantly reduced the effect of both myotoxins, whereas indomethacin only affected edema-forming activity of myotoxin III. It is concluded that (a) these myotoxins are important edema-forming components of *B. asper* venom, (b) enzymatic activity is not a strict requirement to exert this effect, although in the case of myotoxin III it might contribute to its development, and (c) several inflammatory mediators participate in mouse foot pad edema induced by myotoxins.

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P-145Th

STUDIES EXAMINING THE SMOOTH MUSCLE RELAXANT EFFECTS OF VENOM FROM THE INLAND TAIPAN (*Oxyuranus microlepidotus*) IN RAT ISOLATED AORTA.

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The inland taipan (*Oxyuranus microlepidotus*) has been classified as the most venomous snake in the world (Broad *et al.*, 1979). However, research on *O. microlepidotus* venom is limited and focuses mainly on its toxicity. The purpose of the present study was to investigate the relaxation identified in the rat isolated aortae in earlier preliminary studies (Bell *et al.*, 1997). Crude venom (0.1-10 μg/ml) caused dose-dependent relaxation in rat isolated aorta submaximally precontracted with phenylephrine (3-300 nM). Endothelium denudation or a combination of the nitric oxide synthase inhibitor N-nitro-L-arginine (NOLA; 100 μM), the nitric oxide scavenger oxyhaemoglobin (HbO; 10 μM), and the cyclooxygenase inhibitor indomethacin (10μM) did not reduce the relaxant effect. This suggests the relaxation was not endothelial in origin. We then examined a number of smooth muscle-mediated mechanisms. We found the β-adrenoceptor antagonist propranolol (1 μM), the K^+ channel antagonists apamin (1 nM), charybdotoxin (1 nM) and glibenclamide (1 μM) did not affect the relaxation seen in the endothelium-denuded aortic rings, suggesting no effect at β-adrenoceptors or K^+ channels. The possible role of phospholipase A_2 (PLA_2) in the venom-mediated relaxation was also examined. Neither the PLA_2 inhibitor 4-bromophenacyl bromide (4-BPB; 0.72 mM), nor replacing $CaCl_2$ in the buffer solution with Sr^{2+} (10 mM) had a significant effect on the response. In preliminary experiments the venom (10 μg/ml) was found to reverse the contraction to Bay K 8644 (0.3 μM), an L-type Ca^{2+} channel opener, thus suggesting a possible effect on L-type Ca^{2+} channels. The results of this study indicate that *O. microlepidotus* venom causes relaxation in rat isolated aortae primarily by a direct action on the smooth muscle.

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Broad, A.J., Sutherland, S.K. & Coulter, A.R. (1979). *Toxicon* 17, 664-667.

P-146Th

EFFECTS OF SNAKE VENOMS ON TESTOSTERONE AND SOME METABOLIC HORMONES IN MALE RATS

ABTRACT

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The levels of four hormones mainly testosterone, cortisol insulin and thyroxine were investigated in male Wistar rats using radioimmunoassay following acute and chronic treatment of the rats with snake venoms. The snake venoms employed were those of the poisonous type of snake in Saudi Arabia.

A rise in testosterone levels in treated rats was obtained with acute treatment due to vascular permeability and increased testicular blood flow. In contrast, the chronic treatment produced a significant drop in testosterone concentration.

Although the cortisol levels were no different from the controls with acute treatment but showed a gradual rise following chronic treatment. This rise was thought to withstand the stress imposed on the treated rats.

Similar results were obtained for insulin and T_4.

On conclusion injected snake venoms in male Wistar rats affected the secretion levels of the different hormones studied. The effect is highly pronounced with testosterone which is correlated with testicular blood flow.

Keywords: Testosterone, Cortisol, Insulin, Thyroxine, SNake Venoms.

P-147Th

Effects of Russell's viper venom on human erythrocytes in vitro

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The effects of Russell's viper venom (RVV) on human erythrocytes were studied in vitro with respect to packed cell volume (hematocrit), the erythrocyte morphology and the effect of antivenom. Venom at various dosages ranging from 50 ng to 120 μg increased hematocrit significantly. The maximal effect was detectecd at 800 ng of venom. The biconcave erythrocytes shown by scanning electron microscopy became sphero-echinocytes (SEC). Such altered morphology was observed immediately at 1 minute and reached maximum at 30 minutes. There were no morphologic changes when ethylenediamine-tetracetate (EDTA) was used as an anticoagulant or when plasma was substituted by isotonic solution. Phospholipase A_2 at equivalent dose as compared to the venom could also produce the sphero-echinocytosis. Verapamil could not prevent the RVV induced SEC. Although antivenom could not reverse the RVV induced SEC, it minimized these effects.

Conclusions The RVV induced SEC is likely caused by phospholipase A_2. Calcium and some plasma factors are required for this process. Early treatment with antivenom play role in prevention of the SEC which may reduce hypoxic cell injury.

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P-148Th

TERATOGENIC EFFECT OF NAJA HAJE (EGYPTIAN COBRA) VENOM

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There is always a concern about what could happen to the embryo of an envenomated female. Snake venoms contain different biological components. Some of these components may directly affect the placenta and/or cross the placental barrier and affect the developing embryo.

This report deals with the effect of Naja haje venom on the placenta and feti of white albino rat as an experimental model during early periods of gestation. An increase in mortality rate of pregnant animals was observed. Intrauterine fetal death with delayed labour, as well as gross and micro-anomalies were observed. Rebeated sublethal doses injection caused short fore and hind limbs, tail adherent to abdominal wall,atrophy of gluteal region and maceration of skin of the feti.

Histopathological examination revealed cellular infiltration, congestion of blood vessels and area of necrosis and hemorrhage in the placenta and feti organs.

Biochemical changes was also detected. succinic dehydrogenase was elevated in placenta and feti livers and brains indicating disrubtion of the mitochondrial structure. A decrease of the total lipids and phospholipids with an increase of cholesterol content of the placenta might be due to PLA2 present in the venom. DNA increase observed in placenta was probably due to cellular infiltration as documented histopathologically. The RNA decrease could be explained by the activity of ribonuclease U in eosinophils correlated with placental inflammation.

P-149Th

Effect of gamma-toxin from *Tityus serrulatus* scorpion venom on gastric emptying in rats

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The effect of gamma-toxin from *Tityus serrulatus* scorpion venom (GT-Ts) on the gastric emptying of liquids was studied in 176 young adult male Wistar rats (2-3 months of age) divided into subgroups of eight animals each. GT-Ts was injected iv at doses of 25.0, 37.5, 50.0 or 100.0 μg/kg and the effect on gastric emptying was assessed 30 min and 8 h later. A time-course study was performed by injecting 50.0 μg GT-Ts/kg and measuring the effect on gastric emptying at the times 0.25, 0.5, 1, 2, 4, 8, 24 and 48 h post-venom. Each envenomed animal was paired with its saline control and all received a saline test meal solution containing phenol red (60 μg/ml) as a marker. Ten min after administering the test meal by gavage, gastric retention was determined by measuring the residual test meal marker concentration and the animals were then sacrified. A significant delay in gastric emptying was observed only after 50.0 and 100.0 μg of GT-Ts/kg. The responses to these two doses were significantly different only after 8 h post-venom ($p<0.05$, Mann-Wihtney U test). GT-Ts (50.0 μg/kg) significantly delayed the gastric emptying of liquids at all times studied with two peaks responses at 0.5 and 4 h after toxin administration. Additional studies are being performed to investigate mechanisms of the GT-Ts influence on gastric emptying. The gastroparesy observed in the experimental model may improve our understanding of the pathophysiology of vomiting, seen in most cases of severe human envenomation caused by scorpions of medical importance.

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P-150Th

SCORPION STING EPIDEMIC. MONTES COUNTY, SUCRE, VENEZUELA.

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Scorpion stings are potentially fatal, especially for children. Although less frequent than in other countries (91 deaths from 1980 to 1990), this accident is the third cause of death among animal and plant envenomations in Venezuela. There is no detailed epidemiological information on scorpion stings in our country. Montes county had an estimated population of 41891 inh. in 1980 and 50169 inh. in 1996. There is a reference hospital in Cumanacoa, the capital of the county, where all the cases of scorpion stings were registered. We surveyed 17 years data (1980-1996) from the "Morbidity Registry Book" of the emergency service of Cumanacoa's hospital. Nonparametric statistics were used. Differences were considered significant when $P<0.05$. In that period 475 scorpion stings were registered, including 3 deaths. From 1980 to 1990, the annual occurrence of scorpion stings was stable: its median and 95% confidence interval were 17 (14; 21) stung/year. Between 1991 and 1996 the rate rose quickly to 50 (25; 73) stung/year ($P=0.001$). Moreover, 49% of cases occurred from 1993 to 1996. The incidence rate increased from $2.1^0/_{000}$ inh. (1982) to $14.7^0/_{000}$ inh. (1995). The monthly occurrence paralleled the yearly one. It rose from 1.5 (1.0; 1.5) stung/month (1980-1990) to 4 (3.0; 4.5) stung/month (1991-1996) ($P<0.001$). The monthly endemic curve constructed with data from 1980 to 1990, showed that from 1991 to 1996, 74% of the months were epidemic. Severe scorpionic envenomation was stable around 19% in all the period. Compared with other venomous animal accidents, scorpion stings were the most frequent between 1991 and 1996 (39%), with 2 deaths, both produced by scorpions. Our data show a scorpion stings epidemic in Montes county since 1991. Remarkably, a similar increase occurred in other countries (**J. Venom. Anim. Toxins** 1: 51-62, 1995).

P-151Th

LUNG TISSUE ULTRASTRUCTURAL ALTERATIONS ON MICE INOCULATED WITH *Tityus discrepans* (BUTHIDAE) VENOM

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In this work we are presenting the relationships between clinical manifestations that appear as response to the toxic aggression by *Tityus discrepans* venom and the alterations or changes at cellular or subcellular levels that are given in the pulmonary system from this murine model that probably is similar to humans.

The pulmonary tissue shows a partial denudation of its epithelial cells; the basal membrane is irregular and swollen. In the interstice there are fibroblasts with multiple cytoplasmatic projections, as well as abundant extracellular material. Adjacent to the interstice, is appreciated a bronchiolar area; nucleus of these cells presents a disperse chromatin. The mitochondria are edematous and there is loss of the intracellular unions. A clear separation is observed among the cellular plasmatic membranes. At higher magnification is shown a pneumocyte type II with indentations of the perinuclear cistern, as well as the existence of differential populations of mitochondria of condensed conformation, some with autophagic vacuoles in its interior and edematous mitochondria.

Next to the mitochondria are observed multiple lamellar bodies of different sizes, bronchial tissue cells characterized by the transverse presence of their cilia and different electron dense granules. The degenerative sings includes different myelinic images.

P-152Th

Effects of Adrenergic Blockers and Ligation of Spleen Vessels on Hemodynamics of Dogs Injected with Scorpion Venom.

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Objective: In dogs, scorpion venom evokes a rapid rise in cardiac output (CO) that declines below baseline value in 1 hour. The changes in CO are related to changes in venous return (VR). We tested the hypothesis that changes in VR are secondary to sympathetic stimulation, and that initial elevation in VR and CO are secondary to mobilization of blood from the spleen.

Methods: Open chest, instrumented, anesthetized, mechanically ventilated dogs.The effects of α and β adrenergic blockers (9 dogs) and effect of ligation of spleen vessels (11 dogs) following IV injection of venom from *Leiurus quinquestriatus* (0.05 mg/kg) were tested on the determines of VR and compared to effects of venom alone (6 dogs).

Results: Scorpion venom caused marked elevation in CO and mean circulatory pressure (MCP), within 5 min. CO and MCP markedly declined at 60 min. Resistance to venous return (Rv) did not change at 5 min. but increased from 196±50 to 335±102, $p<0.05$ at 60 min. Adrenergic blockade caused attenuation in CO and MCP rise at 5 min. and abolished their decline at 60 min. Rv did not change from baseline to 5 and 60 min. Ligation of spleen vessels prevented CO elevation and attenuated MCP rise from baseline to 5 min. At 60 min. CO markedly declined while MCP returned to baseline. Rv in this group increased from 151±24 to 383±93 at 5 min. and 510±175 $p<0.05$ at 60 min.

Conclusion: Initial rise and late decline in CO and MCP following scorpion venom injection are in part related to sympathetic overstimulation and can be attenuated (initial rise) or completly abolished (late decline) by adrenergic blockade. Elevation of resistance to venous return (Rv) is the most important factor for late decrease in CO. It results from adrenergic stimulation and apparently expresses vasoconstriction and redistribution of blood flow . Initial rise in CO and MCP is explained in part by adrenergic effect on spleen leading to augmented circulatory blood volume.

P-153Th

Tetorodotoxin attracts starfish ("Togemomijigai" *Astropecten polyacanthus*)

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The biological significance of tetrodotoxin (TTX) in TTX-containing animals has recently been discussed, along with progressive development of biochemical studies on TTX.

We intend to elucidate the biological significance of TTX for TTX-containing starfish, premising that TTX is an essential substance for the starfish which is qualified to recognize TTX in diet and therefore looks for TTX- containing diet and takes it. In such a situation, we studied on the attractive effect of TTX for starfish("togemomijigai").

Brief methodology

"Togemomijigai" used in this experiment was caught during from June to September, 1994. The equipment used for this experiment was a Y-shape aquarium(the total length 110cm). Crude TTX which was partially purified was gelled with gelatin and fixed on one side of the aquarium. A test starfish was placed on the base and its behavior was followed by videotaped. In each experiment, the followings were considered.

1. In every experiment, a new starfish was used.
2. TTX preparation was fixed on one side of the aquarium and on the other side, non-TTX preparation. The position of TTX preparation was decided randomly.

Each test sequence was repeated 11 times.

Result and conclusion

We judged a test starfish to be attracted to TTX if it moved toward a TTX containing gelatin from the start. It became obvious that ten starfish were attracted to the TTX. One did not move. This suggests that TTX is not only non-toxic , but in some way perhaps essential for the starfish.

P-154Th

NITRIC OXIDE RELEASE BY *Phoneutria nigriventer* VENOM FRACTIONS IN RAT AORTA RINGS.

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Considering that vascular disturbs were described among the consequences of *Phoneutria nigriventer* spider sting in man we decided to undertake the present study to search for any biological effect of this spider venom in blood vessels. Wistar rats (300-350g) were sacrificed by decapitation and their thoracic aortas were removed and cleaned of surrounding fatty tissue. Two rings (3-4 mm) were taken from each aorta. One of these rings had its endothelium mechanically destroyed. Each ring was mounted in a separate organ chamber containing Krebs-Ringer bicarbonate solution at 37°C, gassed with 95%O_2/5%CO_2 under a resting tension of 1,5g. Mechanical activities were recorded on a model 7 Grass polygraph with the help of force-displacement transducers. After an equilibration period of 60 minutes the rings were exposed to cumulative doses of norepinephrine (NE). Identical procedure was repeated after several washouts and complete relaxation, in the presence of the toxic fraction P_4 of the spider venom (10µg) or of one of its sub-fraction, MAH (muscle-active hydrophilic) (.5µg). In other experiments a 50% maximum contraction was induced with NE and cumulative doses of acetylcholine (ACh) were added to the organ chamber in order to evaluate the integrity of the endothelium and the relaxation magnitude. Identical procedure adding P_4 or MAH instead of ACh was repeated after several washouts. No significant difference was observed in dose-response curves to NE under influence of P_4 or MAH. 10µg of P_4 and .5µg of MAH induced a relaxation of NE precontracted intact rings similar to those induced by 1µM ACh. No relaxation was induced by ACh nor by the venom fractions in endothelium denuded rings. L-NAME (1µM) completely blocked the induced relaxation. We conclude that P_4 and MAH induces liberation of nitric oxide from rat aorta endothelium. This may be one of the reasons to explain some of the vascular disturbs after the spider sting. [Supported by CNPq, FAPEMIG, PRPq/UFMG]

P-155Th

ENDOTOXEMIC-LIKE SHOCK INDUCED BY *L. intermedia* SPIDER VENOM

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Cutaneous inoculation of *Loxosceles spp* spider venoms produces local necrosis occasionally accompanied by systemic reactions. Although at least part of the necrotic skin lesions can be attribbuted to the accumulation of polymorphonuclear cells at the site of the bite, the mechanisms responsible for the systemic reactions are still little understood. The aims of the present study were: a) to analyse the symptomes and tissue lesions following injection of *L. intermedia* spider venom in mice and the emergence of inflammatory mediators in their blood; and b) to investigate the influence of genetic background on the susceptibility of some isogenic mouse strains to the toxic effects of the venom.The estimated LD_{50} were 24.5 µg for C57Bl/6, 17.6 µg for BALB/c, 6.3 µg for C3H/HeJ and 4.6 µg for A/Sn mice. Prostration, acute cachexia, hypothermia, neurological disorders, and hemoglobinuria were the signals preceding death. Accumulation of eosinophilic material inside the proximal and distal renal tubules and acute tubular necrosis were the most common histopathological findings. Death was prevented by previous treatment of venom with specific antivenom serum. The protein F35 purified from the whole venom retained the ability to induce the symptoms of the whole venom. The cytokines TNF, IL-6 and IL-10 and the radical nitric oxide were detected in the serum after venom injection and in the supernatants of lymphonode cell cultures obtained from control or envenomated animals and stimulated *in vitro* with Con A, LPS or *L. intermedia* venom.The profile of cytokine curves were distinct in the high and low susceptible mouse strains and a positive correlation was found *in vivo* between susceptibility to the toxic effect of *Loxosceles* venom and TNF serum levels. These findings indicate that the state of shock produced in mice by whole *Loxosceles intermedia* venom or by its purified fraction, protein F35, mimics the endotoxemic shock; that susceptibility to the systemic effects of the venom varies among individuals and that the pattern of *in vivo* cytokine release also resembles endotoxemic shock. *Supported by CNPq, FAPESP, Fundação Banco do Brasil and Fundação Butantan*

P-156Th

SEX-RELATED DIFFERENCES IN *L. intermedia* SPIDER VENOM

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Inoculation of *Loxosceles sp* spider venom produces local edema, tickening of the endotelium, accumulation of inflammatory cells, intravascular clotting, hemorrhage and necrosis. Generalized systemic effects such as fever, malaise, pruritus and exanthema are very common, while intravascular hemolysis and coagulation, thrombocytopenia and renal failure. The fact that only some cases develop viscerocutaneous loxoscelism has led to the assumption of a possible intrinsic susceptibility of these individuals, although factors related to differences in venom composition at the intra and interspecific levels cannot be ruled out. In order to analyze possible intraspecific differences in the venom of *L. intermedia* spiders we compared venoms obtained from groups of adults males and females. It was verified that females produce two times more venom than males. Although SDS-PAGE analysis has showed similar pattern of protein bands in the two venoms, Western blot and ELISA assays using a polyclonal rabbit antiserum against F35, a 35 kDa protein from *L. intermedia* venom with complement-dependent hemolytic and dermonecrotic activities, revealed a stronger staining of this component in the female venom samples. The venom of females also showed a more potent dermonecrotic and complement-dependent hemolytic activities than venom from males. Therefore, the higher amount associated with a stronger toxicity of female venom as compared with venom from males specimens may contribute in the variability observed in the severity of the accidents caused by *L. intermedia* spiders.

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P-157Th

ONTOGENETIC DEVELOPMENT OF *L. intermedia* SPIDER VENOM

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Loxosceles (*Loxoscelidae*) are ubiquitous spiders in temperate and tropical regions of North, Central and South America, Europe and Africa. The bite of *Loxosceles* species of medical importance produces a local cutaneous lesion, occasionally accompanied by systemic reactions. Envenomation by *Loxosceles* spider have become a public health problem in the last years in Brazil, mainly by the uncontrolled increase of the *Loxosceles intermedia* spider population in the south region. Recently, we have shown that the toxic effects of the *L. intermedia* venom are mainly associated with a 35 kDa protein (F35) which presents a complement-dependent hemolytic activity and is also a dermonecrotic-inducing factor (Tambourgi *et al., J. Immunol. 155*: 4459, 1995). The F35 protein retained the ability to induce the symptoms of the whole venom and also showed a potent lethality for mice (Tambourgi *et al.*, 1997- *Toxicon in press*). In the present study we have analyzed the presence of toxic components during the ontogenetic development of the *L. intermedia* venom. The development of the progeny of *L. intermedia* spiders, breed and kept in our laboratory, were followed during six months in which it was observed four moults. Equal amounts of spider's cefalotorax extracts from the second to the fifth stages were analyzed by Western blot using a mouse serum against *L. intermedia* venom or an anti-F35 monoespecific polyclonal rabbit. Control blots were revealed using normal mouse or rabbit sera. The mouse serum anti-*L. intermedia* venom was able to recognize only a band of 35 kDa in the cefatorax extracts from the four analyzed stages. The same profiles were detected using the anti-F35 serum. In the same experimental conditions it was not observed any reaction using the normal sera. These results show that the production of *L. intermedia* spider venom, including the F35 protein, its main toxic component, starts early during the ontogenetic development and suggesting that young spiders can be also responsible for human envenoming.

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P-158Th

A COMPARISON OF THE PHARMACOLOGICAL ACTIVITY OF VENOM FROM THE MALE AND FEMALE WHITE-TAILED SPIDER (*Lampona cylindrata*).

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Bites by the white-tailed (*Lampona cylindrata)* spider have been implicated as a possible cause of necrotic arachnidism in Australia (Gray, 1989; Skinner & Butler, 1995). A preliminary study by Korszniak (1993) suggested that the venom from the male and female spiders have different pharmacological activities. Therefore, the aim of this study was to conduct a comparison of freeze dried venom gland extracts from male and female white-tailed spiders. In the guinea-pig isolated ileum, venom (2.5-10 μg/ml) from the male spider caused dose-dependent contractions. The response to 5 μg/ml venom was blocked by the histamine H_1-receptor antagonist mepyramine (0.5 μM). In contrast, venom (5-10 μg/ml) from the female spider had no contractile activity in this tissue. In the anaesthetised (pentobarbitone, 100 mg/kg, i.p.) rat, venom (50-100 μg/kg, i.v.) from the male spider caused a dose-dependent pressor response, while venom (up to 400 μg/kg, i.v.) from the female spider had no apparent effect on mean arterial pressure. In the rat isolated vas deferens, both male (10 μg/ml) and female (50 μg/ml) venom caused a decrease in electrically-evoked (0.5 Hz, 100V, 0.3 ms) twitches. A fluorometric assay for the detection of histamine (Shore *et al.*, 1959) indicated a histamine content of 5% (dry wt.) in venom from the male spider, and 0.01% (dry wt.) in venom from the female spider. The results of this study suggest a difference in the histamine content of the venom from male and female white-tailed spiders. Furthermore, the presence of histamine in venom from the male spider accounts for some but not all of the observed pharmacological activity.

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P-159Th

CHANGE OF MUSCLE FIBER TYPE IN FAST AND SLOW SKELETAL MUSCLES INDUCED BY ACL MYOTOXIN

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To study the response of individual muscle fiber types to snake venom PLA_2 myotoxins, we have tested the effect of ACLMT (ACL myotoxin from *Agkistrodon contortrix laticinctus*) on *soleus* and *gastrocnemius* mice muscles. All animals received 5 mg of ACLMT/kg into the subcutaneous lateral region of the right hind limb, near the Achilles tendon. The contralateral muscles were used as control. Frozen muscles were cut at medial region in Cryostat and alternate serial cross-sections stained with toluidine blue and for acid phosphatase, myofibrillar ATPase activity, succinate dehydrogenase and acetilcholinesterase. Signs of fiber injury were identified, three hours after ACLMT injection, in both muscles. Three days after, these muscles showed clusters of recovered muscle fibers. Twenty-one days after *soleus* and *gastrocnemius* presented only chronic signs of damage as split fibers and centralized nucleus. By m-ATPase reactions was possible to identify that both muscle fiber types I and II were injured in both muscles by ACLMT. Significative increased number of fiber type IIC (17.37 ± 4.11 *versus* 1.0 ± 0.6; p=0.008, paired Student t-test) and decrease of type II (60.7 ± 8.8 *versus* 76.8 ± 10.1; p=0.01, paired Student t-test), suggest muscle fiber type change from type II to type I through type IIC. Although ACLMT is known by its myotoxic activity, the results presented here showed that it can also be used as model to induce axonal remodeling and muscle fiber type change in both fast and slow muscle fibers of mice.

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P-160Th

COMPARATIVE STUDY OF THE CYTOKINES PROFILES INDUCED BY *B. asper* AND *B. jararaca* ENVENOMATION IN MURINE MODEL

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Envenomation by snakes from the *Bothrops* genus causes in man and in experimental models pathophysiological changes characterized by local and systemic reactions including impairment of blood coagulation, cardiovascular system alteration producing hipovolaemic shock, hemorrhage and renal failure. In the present study we compared the toxicity and the kinetic of cytokines and NO release in the serum induced by injection of *Bothrops asper* (VBa) and *Bothrops jararaca* (VBj) venoms into BALB/c mice. Lethal toxicity was assessed by i.p. injection of several doses of VBj or VBa into male BALB/c strain mice. LD_{50} of both venoms were calculated according to the probit method. The systemic profile of cytokine release was evaluated in mice sera at several time intervals after injection of one LD_{50} of VBj or VBa. The estimated LD_{50} by i.p. route were 2.0 μg/g for VBj and 3.7 μg/ for VBa. Prostation, acute cachexia and hypothermia were symptoms preceding death. The pattern of serum levels of cytokines upon VBj injection was the following: the maximum peak of IFN-γ was detected at 2 h while peaks for TNF-α and IL-6 were attained at 18h and 6 h, respectively. A positive correlation between high serum levels of TNF-α and VBj-induced mortality was also detected. The cytokines profiles detected in sera of VBa injected mice were: a maximum peak of IFN-γ at 4 h with a second peak attained at 18 h. Peaks of TNF-α and IL-6 were reached at 18h and 6 h, respectively. In addition maximal levels of IL-6 preceded a complete disappearance of TNF-α from sera after VBj or VBa injections. The maximal levels of NO in serum were detected at 2 h and 4 h after injection of VBj and VBa, respectively. Altogether these findings indicate that VBj and VBa envenomations cause a marked release of cytokines and NO in the serum. Since cytokines contribute to the development of shock in a number of pathophysiological conditions, they may play a relevant role in the systemic toxicity caused by bothropic venoms. *Financial support FAPESP; Fundação Butantan.*

P-161Th

EFFECTS OF EQUINATOXINS FROM *ACTINIA EQUINA* ON FISH *IN VIVO*

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Sea anemones from the species *Actinia equina (L.)* produce toxic polypeptides equinatoxin I, II and III, which are lethal in mice (LD_{50} = 23, 33 and 83 μg/kg, respectively) [1]. Surprisingly little is known about the effects of equinatoxins on fish and other marine organisms. Since the principal mechanism of the lethality of equinatoxin in mammals is cardiorespiratory arrest [2, 3] the aim of this study was to investigate possible effects of equinatoxins on gills and on cardiovascular system in fish. Equinatoxin II (N=5) or equinatoxin III (N=5), was applied to the bath in 5 x 10^{-9} M final concentration, and the behaviour of the fish as well as the blood flow through the gills was monitored. In 15 ± 5 sec. after the application of equinatoxins uncontrolled movements of fins were observed, suggesting neurotoxic action of the toxins. Within the first minute after the application the toxins produced a strong and long-lasting vasoconstriction, seen as pale gills *in vivo*. The heart rate was not affected. Investigation of blood samples taken directly from the heart revealed no haemolysis, which is contrary to the findings in mammals. Further experiments were therefore performed on samples of fish blood incubated with 10^{-8} M equinatoxin II, which is known to produce complete haemolysis of bovine, sheep and human erythrocytes, but still there was no effect on fish erythrocytes. Plasma K^+ concentration and hematocrit remained unchanged. The results allow the conclusion that equinatoxins produce vasoconstriction in gills, which is caused either by the direct action of the toxin on the vascular smooth muscle, or through its action on endothelial cells. Haemolysis is not essential for the toxic action of equinatoxins.

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P-162Th

AFRICANIZED HONEY BEE ATTACK BEHAVIOR AND CLINICAL CONSEQUENCES IN HUMANS AND DOGS: EXPERIENCES IN USA

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Africanized honey bees (*Apis mellifera* near *scutellata*) in the warm temperate areas across Texas to California exhibit aggressive patterns similar to those experienced in other parts of the Americas. Our goals were: 1) to determine the nature of the attack on the victim; 2) to predict from fatal cases the outcomes of attacks based on numbers of stings received; and 3) to understand the basis of the unusual deaths of all five humans fatally envenomated in the USA. The sting numbers received by four dogs fatally attacked by Africanized bees were counted and their locations on the body were recorded. The dogs received from 543 to 3305 stings, which corresponded to 27 to 89 stings per kilogram body weight, and over 75% of all stings were delivered to the facial areas, particularly around the snout and eyes. Based on these experiences with dogs and laboratory experiments with mice we generated the following "Rule of Thumb" for bee envenomations: < 14 stings/kg in not fatal; 18 stings/kg is the LD_{50} envenomation; > 22 stings/kg is fatal. We applied this rule to the five human fatalities recorded in the USA and discovered that none of them received nearly enough stings to have died from normal toxinological causes, because they only received from 50 to 300 stings. Rather, they apparently died of causes secondary to the envenomation. All victims were elderly (66, 72, 82, 88, 96 years old) and possessed underlying pathological conditions that appeared to become fatal subsequent to the stinging attack. We conclude that because bees specifically target the vital life-support areas of the face, these areas must be protected during an attack. Moreover, in addition to anaphylactic and toxic reactions to bee stings, a third category of death from bee stings we call "catalytic reactions" should be recognized and treated.

P-163Th

ANTI-CHOLINESTERASE ACTIVITY OF 3-ALKYLPYRIDINIUM POLYMERS, ISOLATED FROM THE MARINE SPONGE, *Reniera sarai*

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Large polymeric 3-alkylpyridinium salts have been isolated from the marine sponge *Reniera sarai*. They are composed of C_4-pyridinyl-C_4-repeating subunits, polymerized head-to-tail and exist as a mixture of two main polymers (poly-APS) with molecular weight without counterion of about 5520 and 18900, according to MALDI-TOF spectrometry. Polymers form large water-solvated supramolecular structures with an average hydrodynamic radius of 23±2 nm and therefore cannot be separated with size-exclusion chromatography. Poly-APS are potent anti-cholinesterase agents, in addition they show hemolytic and cytotoxic activities. The inhibition of acetylcholinesterase by poly-APS shows an unprecedent inhibition pattern composed of three different inhibition kinetics. We tentatively propose a possible model of protein - inhibitor interaction. There is first a reversible binding which takes place at the best affinity site - the peripheral anionic one. Then, the polymeric structure of the inhibitor allows the binding and stabilization of other pyridinium residues at other lower affinity binding sites at the enzyme molecule. The first binding favors the binding to other sites leading to an enzyme-inhibitor complex apparently irreversibly linked end ending in their precipitation.

P-164Th

STORAGE INFLUENCE ON STONEFISH VENOM COMPONENTS ACTIVITY

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Fish venom activity varies according to experiments. In fact, authors do not always mentioned the precise conditions of venom storage. In this study, we compare activity (blood pressure in rats or toxicity of intracerebral injections in mice) of (A): freshly obtained venom in buffer from living fish (*Synanceia verrucosa*); (B, C): lyophilised, diluted venom in buffer and stored one day at -28°C or not; (D, E): just purified verrucotoxin (VTX) (D) or stored one day at -28°C (E). Norepinephrine (NE) presence is screened by HPLC with electrochemical detection.
In rats, A induces a transient hypertension followed by a marked hypotension; the first effect may be linked to about 1.2 μg NE /mg protein in sample venom, the latter to the presence of VTX in venom, as VTX (D) results in an immediate dose-dependent hypotension; this hypotensive effect decreases after storage at -28°C (E), whilst VTX is degraded as shown by chromatography. Neurotoxicity is appreciated by venom-induced seizures always followed by death. Freshly prepared venom (C) injection (0.01 to 0.125 μg protein /g) in mice triggers violent crisis for the highest dose (n = 6) and death within less than 1 hour. After storage one night at -28°C (B), no discernable effect is registered with doses between 0.1 to 0.2 μg/g (n = 5). Only one mouse died within 1 hour with 0.48 μg/g.
Toxic protein(s) in diluted venom samples cannot be stored. The activity may even be inverted: with *Synanceia trachynis* venom, Wiener (1) obtained a marked blood pressure increase in dog after venom storage few days at 4°C. This does not excluded the presence of a factor inducing acetylcholine release (2-4). The same results observed with degraded venom by distilled water dilution and storage at -75 and -80°C), lead to demonstrate the complexity of the hypertensive response (5).
The storage conditions reveal that many components, present in the same venom, are implicated in observed biological response.

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P-165Th

STONEFISH VENOM NEUROTOXIC EFFECTS

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Crude venom of the stonefish *Synanceia verrucosa* induces respiratory and cardiac failure, but death is most often due to neurotoxic clonic or phasic symptoms. The purified lethal fraction, the verrucotoxin, acts through GK_{ATP} channels in frog heart, demonstrated by electrophysiological studies (1). K^+_{ATP} channels are present with high density in the *Substancia nigra* , the *Globus pallidus*, the *Cerebellum* and the *Hippocampus*. The aim of this study is to examine rodent brain response to venom: *(i)* observation of mice after intracranial injection of 0.04 to 0.083 μg proteins /g; *(ii)* EEG and behavior after intracerebroventricular administration of 5 to 50 μg proteins by rat.

In mice, with 0.04 μg /g no appearent effect is evidenced (n = 4), upper doses being all lethal (12 - 60 min; n = 6) with constant symptoms such as ataxia, circling movements, partial or complete reversible limbs paralysis; after a period sleep-like, rolling, scratching and incoordinate movements occur, prior to violent clonic seizure conducing in few seconds to death.

In rats, venom is able to induce seizure (20-50 μg; n = 6/9) terminating in death (15-20 min after dosing; n = 4) for the highest doses. The other animals are rather prostrated or displayed motor disturbances such as circling and incoordinate gait. In animals surviving up to 24 h (n = 2), no microscopic lesions are detected in brain but lungs contain multiple haemorrhagic foci. Venom-induced rotation may be also observed at lowest doses (5-10 μg; n = 2). The effects reported here suggest a contribution of striato-nigral (with dense distribution of ATP-sensitive K^+ channels) pathway in venom-induced disease.

In the brain, the functional role of K^+_{ATP} channels is not yet understood. They participate in the modulation of many neurotransmitters release such as GABA-release in *Substancia nigra* (which plays an important role in seizure disorders) or glutamate release in *Hippocampus* where they constitue a link between ischemia and the development of seizure.

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P-166Th

THE INFLUENCE OF CHOLESTEROL IN MEMBRANE SELECTIVITY OF ANTIMICROBIAL PEPTIDES FROM BRAZILIAN FROG *Phyllomedusa distincta*.

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For the last decades, amphibian skin peptides have been the object of intense interest of many research groups and pharmacological companies due to their extraordinary rich variety of biological activities and biotechnology applications.

Peptides were obtained from adult specimens of *Phyllomedusa distincta* captured at Itanhaem, Sao Paulo State, Brazil.

The extraction was performed by manual compression of the granular glands of *P. distincta* and freshly collected in distilled water as a crude extract. The extract was filtered, frozen and lyophilized. Two mg aliquots of crude extract was submitted to a semi-preparative reverse-phase chromatography using a Bio-Rad Hi-Pore RP-318 (250x10mm) column and linear gradient from 0 to 100% of acetonitrile containing 0.1% TFA over 70 min. The final purification was performed using a Vydac 218 TP 54 (0.46x25 cm) column, producing high pure peptides.

The antimicrobial test and the MIC determination were done by the microdilution method. Five peptides showed antimicrobial activity at micromolar concentrations against gram-negative and gram-positive bacteria.

The capacity to produce hemolysis, of five antimicrobial peptides, was tested using "O" positive human erythrocytes. None of the peptides produced hemolysis until 100 µg/ml (except the peptide L which required 50 µg/ml).

To verify the influence of cholesterol in the selective activity showed by peptides, we produced LUVs (Large Unilamelar Vesicles) of phosphatidylcholine with different cholesterol concentrations. Our results demonstrated that the cholesterol act as an inhibitory lipid in the membranes, decreasing the liposome lysis by *P. distincta* peptides.

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MEXICAN INSTITUTE OF SOCIAL SECURITY: EPIDEMIOLOGICAL DATA ON SCORPION AND SNAKE ACCIDENTS AND THEIR TREATMENT.

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The Mexican Institute of Social Security (IMSS) provides health services to about 40% of the Mexican population. From 1990 to 1996, the IMSS registered 372,181 cases of scorpion stings, equivalent to an incidence of about 150 cases per 100,000 persons covered by the IMSS. The State with the highest rate was Morelos; in 1996, its morbidity rate was 2,698 cases per 100,000. Specific treatment with purified $F(ab')_2$ fragments (Alacramyn®) was used in 76% of the patients. The immunotherapy was not only highly efficient but also safe, since no cases of life threatening acute reactions or serum sickness were observed. Most of the fatal cases (92) can be attributed to either lack of the antiserum or delay from the patient in seeking medical help.

During the 1994-1996 period, the IMSS recorded 1,961 cases of snake bites with 12 deaths. Again, the use of purified $F(ab')_2$ fragments (Antivipmyn®) was of paramount importance in their treatment, not only to save lives but also to limit necrotic sequelae.

Detailed epidemiological data and guidelines for treatment will be presented.

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